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Characterising personality traits in cattle using biotelemetry systems

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Declaration

I declare that I have composed this thesis. This is my own work and any assistance has been duly acknowledged. The work described has not been submitted for any other degree or professional qualification.

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Abstract

On-farm assessment of cattle health, behaviour and welfare is often a logistical challenge but necessary for ensuring high standards of welfare and production. Recently, technological advances in engineering have allowed the mass manufacture of biotelemetry systems for use in research and industry. A commercial dairy farm may now have many different systems recording information about each individual animal in the herd. One such biotelemetry system is the collection of activity data via activity monitors. These devices were initially used by farmers to detect oestrus bouts through the resulting rise in activity and over the years have been improved to collect highly accurate and specific data about lying, standing and steps recorded over long periods of time. Long term, unobtrusive recording of individual cattle activity patterns is now becoming a reality on several farms. This raises the possibility of utilising sensors to remotely quantify aspects of cattle behaviour and welfare across different farms relatively quickly, allowing for the improvement of management and breeding strategies. Before this can be achieved, there needs to be a solid understanding of how behaviour affects activity patterns and how such data should be handled. In this project, the IceTag (IceRobotics Ltd., South Queensferry, UK), was used as a biotelemetry system for recording the activity of cattle. The IceTag is a tri-axial accelerometer activity monitor with a sample rate of 16Hz which has been shown to be sensitive (i.e. few false negatives) and specific (i.e. few false positives) when recording lying and standing behaviour on adult cattle. Cattle's individual variation in behaviour was used as a case study to investigate the usage of this type of biotelemetry system. There were two phases to the study. In the first phase, the capabilities and limitations of the IceTag sensor were investigated. This

involved assessing the extent of behavioural reactions to the IceTag in cattle. The behaviour of 28 lactating dairy cattle at the SRUC Dairy Research Centre was assessed for an adverse behavioural effect of the tags. The results of this study recommended a period of 48 hours from attachment before cattle grew accustomed to wearing the tag. Following this, the capabilities of the tags were assessed. Activity traits calculated directly from the tag and derived from tag data were analysed with respect to performance in four short term tests of temperament in 67 beef steers at the SRUC Beef Unit. From this work, the good repeatability of activity traits including average bout length, daily MotionIndex and daily step count encouraged their further usage. Steers which responded fearfully in a temperament test had higher MotionIndex in the home pen (r_s = 0.35, P = 0.004) and steers which were more capable of displacing other steers at feeding stations also had longer average standing bouts (r_s = 0.26, P = 0.036) and were more variable in their total daily standing duration (r_s = 0.27, P = 0.030). This suggested that fear and sociability related behaviours can be detected through analysis of activity patterns. This work was continued at Wageningen University's Dairy Research Centre where activity was recorded in over 100 dairy cattle. Activity recorded over a forty day period could explain some of the variation in behaviours seen during a subsequent fear test, but not in a social motivation test. The trait 'neophobia' was associated with more lying bouts and a greater variation in lying bout duration in dairy cows (R^2_{adj} = 0.15, $F_{3,75}$ = 5.32, P = 0.002) and bold cows also showed less variation in their lying bout durations (R^2_{adj} = 0.11, $F_{2,75}$ = 5.63, P = 0.005). In conclusion, remote sensors are a useful addition to the ethologist's toolbox, enabling researchers to gain some insight into how fearfully a cow may react without assessing this through on-farm

behavioural testing. Moreover, this work has found that the effects of personality which can be observed in behavioural testing can also be observed in spontaneous behaviour in the home pen away from testing environments. Biotelemetry systems can be utilised as a welfare assessment tool as they record repeatable activity traits which relate to underlying behavioural dimensions linked to the cow's behavioural response to stimuli.

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List of Abbreviations

AI	Aggression Index
CS	Crush Score
DI	Displacement Index
DIM	Days in milk
DMI	Dry matter intake
DNLB	Daily Number Lying Bouts
DNSB	Daily Number Standing Bouts
FFM	Five Factor Model (of personality)
FS	Flight Speed
g	Grams
g	Common use. Acceleration relative to freefall (SI unit is ms^{-2})
HAP	Human Approach in the Passageway
Hz	Hertz, unit of frequency, equal to one cycle per second
Kg	Kilograms
m	Metres
MI	MotionIndex™
ms^{-1}	Metres per second
NANO	Novel Arena-Novel Object
SOC	Social Runway Test
SD	Standard Deviation
SEM	Standard error of the mean
TMR	Total Mixed Ration

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Chapter One

General Introduction

1.1 Introduction

The modern dairy industry has undergone significant changes since the 1960's. This so-called 'livestock revolution' has been characterised by production systems which have become more intensive, with larger farms housing more animals in more confined systems, with concentrated diets being fed to increase production traits (Fraser 2008). This new form of agriculture brings with it a whole new set of challenges regarding animal welfare (Mench 2008). An animal must have both physiological and behavioural capabilities to cope with these systems. An individual animal's disposition to respond in a certain manner to stimuli may be termed the individual's personality (Gosling & John 1999) and the composition of personalities within a social group can significantly influence group behaviour (Uher 2008). There are many terms used in the literature to discuss individual behavioural variation and this has caused some confusion. The differences and similarities in terminology will be discussed in Chapter Two. For the sake of consistency, this chapter will discuss individual personality and the related concept of temperament (based on behavioural testing). For the purposes of this chapter only, these terms should be considered interchangeable. The individual's personality can affect how the animal copes and interacts with its environment (Manteca & Deag 1993a) and a greater understanding of the individual's differences will contribute to improved animal welfare (Manteca & Deag 1993b). The behavioural characteristics of cattle, such as their fearfulness, sociability, aggressiveness, etc. are important aspects of how the animal adapts to the farmed environment. In modern agriculture, cattle are kept in a wide range of environments, from highly intensive indoor systems to large extensive outdoor systems. A thorough understanding of how these traits affect the animal's life can

improve the welfare of the herd but also provide mechanisms for which to select animals which are best suited for the current environment.

In this chapter I will discuss cattle welfare and how it relates to production and personality in cattle. I will then discuss how new biotelemetry systems have been applied in farming, ecology and ethology, particularly with regard to how these techniques have been adopted by the cattle farming industry. I will discuss the possibility of harnessing this new trend as a source of data on cattle behaviour by giving an overview of biotelemetry systems, their current uses, limitations and unintended consequences. Finally I will give an overview of how novel applications of biotelemetry systems may aid the collection of behavioural data and contribute to the understanding of cattle personality.

1.2 Challenges to cattle welfare

Traditionally, animal welfare has been seen as the absence of negatives, such as the absence of illness or injury (Von Keyserlingk et al. 2009), however more recently animal science has also begun to investigate how the animal perceives its environment, how it adapts to living in an 'unnatural' environment and the presence of positive welfare indicators such as play and social licking in cattle (Fraser 2008; Napolitano et al. 2009). 'Natural' environments are difficult to define. Historically cattle have been used for a wide variety of purposes. They have been used for meat production, dairy production, for leather, for labour as draft oxen, and even their faeces and methane outputs have been utilised as fuel (Vigne & Helmer 2007). It is estimated that the ancestral aurochs were first domesticated 10,500 years Before

Present (Bollongino et al. 2012) and that this ancestral pool could have been as small as around eighty female aurochs. Bollongino et al. extrapolated that there was likely a single domestication event with this small founder population in a few Neolithic villages and points to the archaeological sites showing earliest evidence of auroch domestication being less than 250km apart as evidence of this. Bollongino et al. also theorised that attempting to manage the wild auroch was complicated by their aggression, territorial instincts and wariness of humans. These behavioural traits would seemingly make the auroch unsuited for domestication but their utility, particularly their dairy products, motivated early farmers to overcome these behavioural barriers to domestication (Vigne & Helmer 2007). We no longer live or farm by Neolithic standards. The global cattle population is predicted to increase from 1.5 billion animals today to 2.6 billion animals by 2050 (Foresight 2011). This increasing demand for produce has led to increasingly intensified farming systems for both beef and dairy cattle. Dairy farmers in the UK, for example, have increased their average herd size from 72 animals per herd in 1996 to 112 animals per herd in 2008 (DairyCo 2010) and selection for high yielding cows and changes to the management systems have increased the average milk yield from 5,151 litres per cow per year in 1990 to 7,533 litres per cow per year in 2011 (DairyCo 2012). Cattle are now living in larger groups with increased competition for food and resources and may be in close contact with humans every day for management purposes. It is a system very unlike that in which they were first domesticated.

It is possible for cattle to adapt to modern systems. In domesticating cattle we have, intentionally and unintentionally, bred for a more docile animal which is more

accepting of humans, for reduced maternal protectiveness in some breeds of dairy cows and increased the behavioural plasticity of cattle in general, making them more adaptable to the wide range of environments humans took them to (Mignon-Grasteau et al. 2005). However there are still behavioural challenges which face cattle in modern management systems.

In the dairy industry, larger herds with more, higher yielding animals is one example of how changing production systems present challenges to cattle welfare. These challenges can be physiological. In dairy cattle, selection for high milk yield has resulted in a dairy cow which lactates for a long period, resulting in high physiological stresses impacting energy balances, fertility, udder health and milk composition (Veerkamp et al. 2003; Ouweltjes et al. 2007). In dairy cattle, there has also been a decline in fertility. The decline has been significantly associated with disease such as mastitis and lameness (De Vries et al. 2011) and 'routine' surgeries and hormone treatments (Dobson et al. 2001), incidences of either can reduce fertility rates. Incidentally, this is an example of how poor welfare in the form of disease and pain can also impact production, as fertility is related to milk yield so a negative effect on fertility will reduce yield. Freedom from pain and disease is a fundamental aspect of animal welfare (Dawkins 2006a) and pain and disease are clearly negative impacts on cattle welfare. The metabolic stresses of producing milk can be dramatic. A cow in an intensive system will live approximately 5-6 years as opposed to the 20-25 year lifespan of a cow in a less intensive system (Phillips 2001). The increased intensification in production can impact behaviour as well as the animal's physiology. Large herds with hungrier animals will result in increased

competition at the feedface which the cattle find stressful and impacts many aspects of their feeding behaviour (De Vries et al. 2004; Hosseinkhani et al. 2008). When it is not possible for all animals to access the food resource at the same time competition must occur. A dominance hierarchy will be established with more dominant animals having better access to the food resource (De Vries 2006). Subordinate animals can find these situations stressful and make suboptimal choices to avoid conflict. For example, subordinate dairy cows will choose a low-quality food in a choice test to avoid feeding next to a dominant cow (Rioja-Lang et al. 2009). In the home pen, this avoidance behaviour can result in many disrupted meals for cows which are subordinate, while dominant cows will have longer, fewer meals (Rioja-Lang 2009). Competition is undoubtedly a natural behaviour, but one that can cause social problems and suboptimal choices, which illustrates how natural behaviours in an environment which is ill suited to their expression is having a negative impact on animal welfare (Dawkins 2006a).

In this instance the descriptors we use to describe individual behavioural traits such as ‘dominant’ and ‘subordinate’ relate to how the cow responds in competition with other cows for a resource. However, it has been suggested that dominance is a personality trait in animals (Gosling & John 1999), affecting how the individual responds to stimuli. In cattle, the other members of the herd are a stimulus the individual responds to, either in competition for a resource, such as food or lying space, or socially through mutual grooming sessions. There are other personality traits which relate to how the individual responds to other herd members, such as aggression and sociableness (Gosling & John 1999). Attempts to breed for

robustness (i.e. reduced lameness, mastitis and improved fertility rates) in dairy cows have been associated with more aggressive animals which have lower levels of social synchrony within a herd (Lawrence et al. 2009). The attempt to improve dairy cattle welfare by breeding for disease resistance had the unintended side effect of increasing the aggression displayed in the animals, another welfare issue. Aggression allows the individual to defend itself and resources and is clearly a natural behaviour, satisfying the animal's need to defend a resource, but must be considered carefully in a welfare context (Bracke & Hopster 2006).

Another natural state for animals is that of fear. Fear is important for cattle as prey animals however it is an unpleasant and stressful emotional state (Boissy 1995). In intensive indoor systems, cattle are protected from most predators. However the cattle can still have a fearful response to stimuli they encounter regularly, even if the stimuli are not harmful. For example, handling by humans is known to be stressful for some cattle, provoking a fearful response. We can improve the welfare impacts of this fear response by adapting handler skills and handling facilities. Beef cattle raised in close contact with humans, and so habituated to their presence, are less unnerved by human observers in novel object tests and in unfamiliar contexts (Boivin et al. 1998). This study also found that cattle were able to generalise their response to a human throughout different situations, e.g. a bad experience in the barn will result in wariness in the field. This ability to learn and to generalise fearfulness is very important for the animal's day-to-day life and can significantly change the

behaviours shown. Cows will easily learn to discriminate against a negative handler and will approach a positive one more easily (Munksgaard et al. 1997).

1.2.1 Animal welfare and productivity in cattle

In the previous section on animal welfare it was discussed how 'good' welfare is more than the relief of negative emotional states such as stress, pain and fear. In agriculture, improvements to animal welfare are sometimes linked to improvements to productivity, such as improved disease incidence being related to higher milk yield. Understanding how behaviour and welfare are linked can greatly improve animals' welfare. It is particularly pertinent for human-animal interactions as personality traits such as fearfulness can prompt an animal to have an extreme reaction to something they may experience every day. In one study, Breuer (2003) treated one group of heifers positively and another group negatively. The negatively treated group showed increased blood cortisol levels when presented with humans, showing stress due to poor handling experiences. Here the behavioural variation between the two groups was manipulated experimentally, but differences in levels of fear also exists within a 'natural' population, consistent within individual cows and varying between them (Boissy & Bouissou 1995). Dairy heifers which show more fearful behaviours in testing environments have inhibited milk ejaculation on their first milkings and higher cortisol levels (Van Reenen et al. 2002). This is an instance where the higher stress response in some individuals caused by the personality trait of fearfulness has an impact on production, although these differences may not be significant after the individual has grown more experienced in the milking parlour.

There is also a relationship between personality traits and production in traits in beef cattle. Voisinet et al. (1997b) studied beef cattle breeds and found that the calmer animals in a handling environment put on weight more easily, resulting in a higher yield for the farmer. Similarly, easily agitated animals produced meat tougher than established food thresholds (Voisinet et al. 1997a). Fear behaviours, such as agitation and escape attempts, displayed in handling areas have been linked to poor meat quality in beef steers (Kadel et al. 2006; Müller & von Keyserlingk 2006; Turner et al. 2011b). The genetic correlations of these traits have raised the possibility of breeding for less fearful cattle (Burrow 1997; Reverter et al. 2003; Kadel et al. 2006). The link between variation in production and variation in how the individual responds to stimuli, i.e. personality, has already been made. To investigate this further, we need to be able to assess behavioural variation across large numbers of animals.

1.2.2 The advantages of assessing personality remotely

In the example of breeding for robust cows (Lawrence et al. 2009) the structure of behavioural variation within the cattle population was changed, inadvertently, by the selection process. It has been suggested that behavioural traits should be included in breeding programs (Kadel et al. 2006; Gibbons et al. 2010; Turner et al. 2011a) in attempt to reduce the possibility of welfare challenges and also to improve production. It is essential that we more fully understand how personality affects how the animal perceives its environment and how this perception affects its welfare before attempting to breed for personality types we perceive to be advantageous. In order to understand how personality affects cattle welfare there is a need for large

numbers of cattle to be assessed in a standardised manner across different management systems. As ever, the need for more data is a driving force in the field (Lawrence 2008). Unfortunately, the behavioural tests for personality are time consuming (Forkman et al. 2007; Windschnurer et al. 2008). Is it possible to remotely assess personality in cattle through behaviours recorded with biotelemetry systems? If so, welfare could be assessed quickly, without laborious on-farm testing and it would be easier to undertake large scale assessments. As biotelemetry is being more widely adopted in agriculture, generating large databases (Rushen 2012), this project will investigate to what extent some behavioural tests of personality in cattle relate to spontaneous behaviours of activity in the home pen. This would be the first step in assessing personality remotely.

1.3 Personality in cattle

Within a herd of cows there will be variation in the behaviours displayed by individuals (Van Reenen 2012). Not all cows will display the same aspects of the species' behavioural repertoire to the same degree. This often happens in clusters of similar behaviours. For example, all cows must compete at a feeding area but not all cows will show the same levels of aggression to other cow. Some will be consistently less aggressive and others more aggressive (Gibbons et al. 2009b). Cattle have the capacity to show fear in response to an unexpected stimuli (Forkman et al. 2007). However the levels of fear displayed by individuals towards the same stimuli will vary and again this variation is consistent within the individual (Gibbons et al.

2009a). Therefore we speak of ‘aggression’ and ‘fearfulness’ as being personality traits in cattle.

How many personality traits exist in cattle? In studying personality traits in animal species, many ethologists have found five main traits similar to the human Five Factor Model (FFM) of personality (Gosling & John 1999). The five traits in the FFM are commonly referred to as neuroticism (featuring anxiety, depression, a vulnerability to stress and moodiness), agreeableness (featuring trust, cooperation and a lack of aggression), extraversion (featuring sociableness, assertiveness, activity and general positive emotions), openness (featuring intellect, imagination, creativity and curiosity) and conscientiousness (featuring deliberation, self-discipline, dutifulness and order) and these are commonly abbreviated to NAEOC (Gosling & John, 1999). There is some debate as to whether the FFM is appropriate to use in animal species (Uher 2008) or even within all human cultures (Gurven et al. 2012) as many of these labels encompass traits that we might consider to be exclusively human orientated. Gosling and John (1999) found that extraversion, neuroticism and agreeableness were all observed in a range of animal species from primate, non-primate mammals, octopus and fish. They also found evidence of openness in several species and the possibility of two extra dimensions in animal species, activity and dominance. However, the difficulty in interpreting these labels means that these words are often not used to describe the traits in the studies where they are found (Gosling & John 1999).

How then do we know if something is a personality trait? Merely exhibiting consistent individual behavioural variation is not enough for that characteristic to qualify as a personality trait. All animals must sleep, and there is individual variation in sleeping patterns which is related to innate biological differences in the amount of sleep needed, and the affective state of the animal at attaining sleep (Langford & Cockram 2010), yet sleep is rarely spoken of as a personality trait. This is, in part, convention, as there are few guidelines on how to refer to behavioural variation across differing contexts or between individuals and populations. In general, variation in behaviour that is not due to developmental processes (such as age), or gender are known as personality traits. A more detailed investigation into the structure of consistent individual behavioural variation is conducted in chapter two. For the mean time, we will discuss personality as being the *individual behavioural variation in response to environmental challenge which differs consistently from other individuals in the same population* (Uher 2011). This behavioural variation may be adaptive (Wilson 1998).

Within cattle, there is no stated consensus as to how many personality traits may exist. The research tends to focus on those traits which have a clear relationship with welfare, such as fearfulness and sociableness, which may be related to the FFM traits of neuroticism, agreeableness and extraversion. A personality trait must be rigorously validated and found to be repeatable across time (Carter et al. 2012a) and for the purposes of remote assessment, must be able to be observed in a testing environment. The following section will review how personality and temperament is

assessed in cattle and for practical purposes this will give an indication of how many traits are used to describe behavioural variation in the cattle species.

1.3.1 Assessing personality in cattle through tests

Temperament and personality are similar and are often used interchangeably in ethology (Gosling 2001) so this section will discuss personality assessment in cattle and include temperament testing. Distinctions between the two terms will be further discussed in Chapter Two.

Stockpeople and farmers are comfortable with assigning their animals a 'temperament type' (Kilgour & Dalton 1984) and readily acknowledge that their interactions with their animals can change the animals' experiences and personality, for better or worse (Bertenshaw & Rowlinson 2009). The heritability of traits such as 'milking temperament' and 'social dominance' were seen as possible selection criteria to breed cows which were easier to milk (Dickson et al. 1970), making production more efficient. In Dickson et al.'s (1970) study, milking temperament was assessed by asking stockpersons to rate the animals on a 1-4 scale (**Table 1.1).** Note how the cow's perception of the process, as judged by the stockperson, and her levels of stress are essential for classification. The basic assumption was that cows perceive the event differently and have different levels of distress in response to this. This context specific term predicts the cow's behaviour in a milking session and also adds a qualitative aspect to it, poor to ideal. From this it is easy to see why being able to classify animals in such a way is an attractive prospect for farmers looking to breed 'better' animals.

Table 1.1 *Score sheet used by milkers to rate milking temperament of dairy cattle, adapted from Dickson et al. 1970*

The following are descriptions of different levels of milking temperament. Would you read over the descriptions and rate each of your cows according to the category which comes closest to describing her behaviour during milking?	
Description of Temperament	Score
Very quiet; never gives any trouble, extremely docile during milking and preparation; the 'ideal' milker.	1
Stands quietly in stall; not bothered by preparation or milking, but may move frequently, shifting weight from side to side, may flick tail occasionally, gives very little trouble	2
Generally quiet, but moves around a lot; may lift feet occasionally during preparation of milking but not kick, flicks tail frequently or appears restless occasionally.	3
Appears very restless during preparation or milking, kicks at handler occasionally, steps from side to side a great deal, quivers when a hand is placed on her.	4

There are several comprehensive reviews of cattle personality and temperament tests (Waiblinger et al. 2006; Forkman et al. 2007; Windschnurer et al. 2008). They tend to focus on fear-related behavioural responses as this is a welfare issue that has attracted the most research. **Table 1.2** lists commonly used tests which relate to the human Five Factor Model of personality (Wilson et al. 1994; Gosling & John 1999) and which estimated the repeatability of the test. Repeatability is an important aspect of testing a personality trait as we are interested in the consistent behaviour variation, a non-repeatable test may not reflect an underlying behavioural trait. Historically to establish the existence of a trait, repeatability within the individual has been an important component of the research. The repeatability of the trait also infers some information about the heritability of the trait (Boake 1989). Heritability is never higher than repeatability and so a trait with low repeatability is unlikely to be worth

investigating from a breeding perspective. In some instances, heritability has been calculated instead of repeatability and this has been noted in the table.

Many of these tests are regarded as 'gold standards', which in an animal welfare context means they are the best performing tests available. Broadly speaking, a gold standard test is the best available diagnostic or benchmark under reasonable conditions. Waiblinger et al. (2006) discuss how both the repeatability of a test and the validity of a test should be considered when selecting the appropriate test for studying farm animal behaviour. The validity of a test encompasses whether the test's measures are free from systematic errors, is it an accurate, reliable and sensitive measure of animal welfare, does it give relevant information to the research question and does the method have relevance in other situations? The validity of the test is also concerned with whether the test measure reflects what it is supposed to, i.e. does a test of fear actually measure the animal's fear? In this manner it can have convergent validity (conceptually related measures are associated with the measure in question) and discriminant validity (are conceptually unrelated measures independent of the measure in question). For example, Gibbons et al. (2010) demonstrated convergent validity in the creation of their social runway test in dairy cattle by relating it to other conceptually similar tests of sociability such as nearest neighbour distance and herd synchrony. Dairy cattle which were quick to rejoin the herd in the social runway test, assumed to be more sociable, were also more synchronous and had a closer nearest neighbour distance, both also thought to reflect sociability. Discriminant validation is more complex as it requires two conceptually unrelated tests. Waiblinger et al. (2006) give the example of a general fearfulness test

not correlating with a measure thought to reflect pleasant experiences with a human. Boissy & Bouissou (1988) found that human avoidance and responses to non-human stimuli were not related in a group of cattle which had experienced early handling, and Waiblinger et al. (2006) consider this to be an example of discriminant validity between human-avoidance and fear in relation to novelty. Researchers must be cautious when considering what trait they expect a test to reflect. Discriminant validation is particularly difficult to consider when in the field of behavioural syndromes. As Chapter Two will go on to discuss, behavioural syndromes assumes underlying structures of variation which connect behaviours in two different contexts, which may seem to undercut the concept of discriminant validation. In selecting tests for this project, it is important to choose tests which have been validated with previous work and not to create novel tests. As is the nature of scientific progress, in using tests this project will be continually validating them, and may re-evaluate the usage of certain tests in light of the project's results.

Many of these tests, such as reactions to novelty and reactions to humans, reference the same elicited emotions, such as fear. However many of the tests which concern fear eliciting stimuli use different kinds of stimuli, such as novel objects or humans (Carter et al. 2012b). Humans can elicit fearful behaviours but are also capable of eliciting positive behaviours in their interactions with cattle. It is important to comprehend the stimulus presented to the animal, the context in which the test takes place (i.e. at the feedface, in the home pen, in a novel environment, etc.) as they will affect what underlying motivations the animal will be experiencing during testing.

From this body of work, we know that the personality traits in cattle which are repeatable across time relate mainly to how fearful the individual is within a situation and how the animal responds to conspecifics, both in competitive and social situations. While other traits may exist within the cattle population, the relatively well established and validated nature of these tests and traits make them ideal for this project. It is important to use traits that have been well defined to explore the possibility of assessment using remote sensing so this project will focus on fear-related traits and conspecific-related traits only.

Table 1.2 *A summary of tests used to establish individual differences in behavioural variation (referred to as personality or temperament) in cattle where repeatability or heritability as a related indicator was estimated.*

Test	Short description	Trait referred to	Repeatability Estimate	Reference
Social Motivation Test	A runway is set up with a set of companion animals confined at one end. Subject animal is separated from group and latency to return is recorded, as well as other behavioural measures.	Sociability	Estimates for different measures across 3 tests range from 0.39 to 0.54 (repeatability calculated through variance within/between animals)	(Gibbons et al. 2010)
FeedFace Dominance	The number of physical displacements received and given at the feedface is collated for each individual to create an 'index of success'.	Dominance	0.29 (Gibbons et al. 2009b) (repeatability calculated through variance within/between animals)	(De Vries et al. 2004; Rioja-Lang et al. 2009; Gibbons et al. 2009b)
FeedFace Aggression	The number of times an aggressive incident is instigated and number of times aggression is received at the feedface is collated for each individual to give an 'index of aggression'.	Aggression	0.31 (repeatability calculated through variance within/between animals)	(Gibbons et al. 2009b)
Social Confrontation Test	Subject animal is introduced to novel animal in familiar arena. Number and type of interactions with novel animal recorded with and without food resource.	Sociability	Not calculated	(Raussi et al. 2005)
Novel stimuli in familiar environment	Subject animal is presented with a stimulus judged to be novel to the animal in an environment (such as the home pen) where the animal is settled. Object related behaviours recorded.	Fearfulness (Novelty)	Low consistency within animals for reactivity across three types of novel stimuli (W_{29}=0.27, P<0.005) (Gibbons et al. 2009a)	(Herskin et al. 2004; Gibbons et al. 2009a)

Novel Arena Test/Open Field Test	Subject animals are introduced to a novel arena, behaviours exhibited in the arena are recorded.	Fearfulness (Novelty)	Variables constructed from factorial analysis correlate within individuals across time	(Visser et al. 2001; Van Reenen et al. 2004; Van Reenen et al. 2005)
Novel Object in unfamiliar environment	Similar to above, but with inclusion of novel object	Fearfulness (Novelty)	r_s=0.55, P<0.05 (Van Reenen et al. 2005)	
Human Approach (Involuntary)	Human approaches animal and records when animal steps away. Can occur in different areas	Fearfulness (Humans)	Approach in passageway 0.65, approach when animal lying 0.40, approach at feedface 0.27	(Gibbons et al. 2009a)
Human Approach (Voluntary)	Human stands in arena or familiar environment and animal chooses to approach. Latency and behaviours recorded.	Fearfulness (Humans)	Between-experimenter reliability correlations high r_s=0.62, P<0.001 for % of animals in herd that can be closely approached.	(Waiblinger et al. 2003; Windschnurer et al. 2008)
Flight Speed	Speed upon leaving a handling area is recorded as a proxy measure.	Fearfulness (Handling)	Variance explained by animal r=0.51, P<0.001 (Turner et al. 2011b)	(Burrow 1997; Kadel et al. 2006; Turner et al. 2011b)
Crush Score	Behaviour when held in a crush is scored on an ethogram	Fearfulness (Handling)	Variance explained by animal r=0.35, P<0.001 (Turner et al. 2011b)	
Exit Score	Subjective assessment of animal's gait upon leaving handling area.	Fearfulness (Handling)	Reliability of score between days within observer Kappa Coefficient between 0.30 and 0.46 for different days	(Vetters et al. 2013)
Separation Test (1) and Restraint Test (2)	Individual removed from group by a handler and restrained unless aggression shown (1) then separated animal approached and restrained in separate pen and stroked if possible (2). Subjective scores given.	Handling temperament	Heritabilities estimated at between 0.00 and 0.61 (±0.17) for German Angus cattle and 0.00 and 0.59 (±0.41) for Simmental.	(Gauly et al. 2001)

1.4 Biotelemetry systems in agriculture

There are many uses of biotelemetry systems in agriculture such as identification of disease or oestrus detection. In dairy cattle farming biotelemetry systems are becoming more common place with oestrus detection, parlour monitoring for milk flow rates, milk yield and milking time. This has helped to reduce stock person workload. For example, due to the short length of time stockpersons have to observe cattle on modern dairy farming systems and the short duration of an oestrus event, automated methods of detecting oestrus have become available. In a review, Firk et al. (2002) assessed several methods of oestrus detection for sensitivity (proportion of correctly detected oestrus episodes) and specificity (proportion of detected oestrus episodes which were in fact false). The oestrus detection methodologies included visual oestrus detection, progesterone content of milk, changes in milk yield, internal body temperature and milk temperature, vaginal mucus resistance, mounting activity and walking activity as recorded by a mercury-tilt switch pedometer. Of these parameters, activity remotely recorded by pedometer was the single most accurate method of detecting oestrus. This result has increased commercial interest in recording cattle activity for the detection of oestrus on farms. This is an example of how biotelemetry systems can be adopted by the farming industry as they become cheaper, easier to employ and aid in farm management.

As well as oestrus detection, changes in behaviour are being increasingly used in disease detection. For example, long lying times and lying bout variability can be an

indicator of lameness in dairy cattle (Ito et al. 2010), which is understandable as lying removes pressure from affected feet and alleviates pain. The duration of time the individual spends feeding and changes in feed intake can be an indicator of ketosis and chronic lameness (González et al. 2008). The possibility of using biotelemetry systems to identify welfare challenges within groups of animals is an attractive prospect to researchers, particularly as farm sizes grow. The possibility of being able to identify a sick animal automatically via database notification promises reduction in human labour observing animals and the animal's time spent suffering. For these reasons, the use of long term biotelemetry in agriculture is being quickly adopted, particularly by researchers. Rushen (2012) found a sixteen fold increase in the number of articles referencing automation and animal welfare in the previous two decades.

1.4.1 Overview of a biotelemetry system

The Merriam-Webster dictionary defines 'biotelemetry' as 'the remote detection and measurement of a human or animal function, activity, or condition (as heart rate or body temperature)'. Biotelemetry is widely used in animal science with many different kinds of systems available, these are reviewed by Cooke et al. (2004). The basic biotelemetry system records data via a sensor which samples some aspect of an organism that it can quantify, e.g. an electrocardiograph records electrical activity through electrodes and interprets this activity as heart rate. This vague 'sensor and interpretation' definition means that biotelemetry systems can vary greatly. Cooke et al. (2004) classed them into two broad categories, devices which transmit their

signals to receivers, sometimes as far away as orbiting satellites, i.e. GPS systems, and devices which store their data for later retrieval, i.e. data loggers.

Both types of system require some type of sensor and usually a power source. In transmitter-receiver systems, real-time transmission of data, such as a radio wave frequency (as in the case of radio-tags), or a visual display (as in the case of most digital thermometers), require an observer to note a state change. The device has no memory of what it was recording previously. For example, radio tags sample location in an indirect method, transmitting a signal on a distinct frequency which can be received by an antenna. Manual triangulation of the signal provides the location of the tagged animal. Such methods have long been used in wildlife research where direct observation is likely to change the animal's behaviour or direct observation is not possible due to cover or large home ranges. The other kind of biotelemetry system is commonly referred to as a data logger, which does not require an observer to record the data. Instead the data stored on a memory chip. Data loggers can store data on the same device as the sensor (e.g. be animal mounted) or use a transmitter and receiver setup to store data remotely (Hawkins 2004). Animal mounted data loggers without transmitters require the retrieval of the data storage device after the study period. Portable data storage is often costly with regards to power and added batteries can increase the weight of a device, making them more cumbersome. Each biotelemetry system is therefore unique, with different requirements for power, data transmission or storage, and the how robust it needs to

be to remain functional. Similar systems can also be used on a large variety of animals (Shepard et al. 2008).

1.4.2 Commercially available on-farm data generators

Many of these automated systems record measures continuously, creating large pools of data with information on each individual within the herd. This includes robotic milking parlours which collect data on milk yield and quality as well as number of visits and the number of successful milking bouts. Pressure plates such as StepMetrix™ (BouMatic, Madison, Wisconsin, USA) positioned outside a milking parlour record the weight distribution on all four hooves of a cow, at each milking. These kinds of devices rely on a rolling average of weight distribution and torsion measured on the plate per cow to identify when an animal is becoming lame and so by nature generate a large amount of data. Automated feeders such as HOKO feeders (Insentec B.V., The Netherlands) record the number of feeding bouts, feeding bout duration and feed intake of individual cattle. Again this system records data continuously, allowing the farmer to look back over months of feed intake data for each individual animal. As previously mentioned one of the newest and largest markets is surrounding oestrus detection using activity monitors and these too continuously record large amounts of data. Farmers have a range of choices such as the SmartDairy® Activity Module (BouMatic, Madison, Wisconsin, USA) which is a small activity monitor fitted to the cow's neck or leg and stores an activity profile, alerting farmers to increases in activity associated with oestrus episodes. Similarly the Silent Herdsman (NMR, Chippenham, UK) is a tri-axial accelerometer fitted to a

collar on the cow's neck. The device continuously records data, downloading to base stations on the farm in places, such as the milking parlour, again recording continuous activity and alerts the farmer to oestrus events. Alternatively, CowAlert® (Alta Genetics) pairs the IceQube® (IceRobotics Ltd., South Queensferry, UK) with an automated system to alert farmers to oestrus events. Researchers have even more options regarding activity monitoring, such as the popular HOBO® Pendant Data Logger (Onset Computer Corporation, Massachusetts, USA) which has a shorter data-logging lifespan but is very adaptable, being small and robust and with many options for mounting to the animal. With the exception of the HOBO loggers, these devices are all designed for use by for farmers and include in their price the set-up of a database to collect and store long periods of continuous activity data for many individuals. The possibility of exploiting this resource to inform on cattle welfare is a very exciting prospect. However, with little work being done to determine how activity relates to the animal's internal state, or how activity varies between individual cows even within the same management system, the danger is that these databases will be merely be an exercise in data storage (Rushen 2012). In one study using this type of data, individual variation in cow activity patterns varied considerably, with dairy cows within the same herd averaging between 5.9 and 15.3 hours lying in a day (Tolkamp et al. 2010). Why does such variation exist in dairy cattle and does this inform on cattle welfare? Before sensors can be fully utilised as welfare indicators, it is important to understand not only the capabilities and limitations of such sensors, but also some of the driving forces of behavioural variation within and between individual cattle.

1.5 Limitations of biotelemetry systems

The ability to monitor the animal when direct observation is impossible is one of the great advantages of biotelemetry (Altmann 2003). Unobtrusive observations of animal behaviour can be difficult to achieve and many methods exist to hide the observer or to habituate the animals to the observer (Martin & Bateson 1993). The presence of observers may affect the behaviour of an animal, although the extent of the effect is sometimes contested. It is acknowledged that human observers can have an effect on the behaviours that animals display, but debate still arises as to what extent observed behaviours are valid, or are an artefact of the animal's fear of the observer (Crofoot et al. 2010; Lutz & Nevill 2011). Biotelemetry is sometimes seen as a way of circumventing this problem. However, by manipulating an animal by fitting it with a biotelemetry device, we raise the possibility of inadvertently affecting its behaviour, and so we do not truly know what the animal would do if it were not fitted with a device.

The previous section discussed the variation in the size and requirements of different biotelemetry systems. When fitting a biotelemetry device to an animal, one concern is that the device will incur a fitness cost by reducing the ability of the animal to forage, hide itself, compete for a mate or have some other negative effect. This problem was often found in birds in earlier studies utilising biotelemetry. Homing pigeons fitted with transmitters have to work significantly harder to fly than those

without, resulting in a increase in their metabolic rate and decreased flight speeds (Gessaman & Nagy 1988). Spotted Owls which were radio marked had lower fecundity than non-radio marked counterparts (Foster et al. 1992). The authors of this study speculate that this was possibly due to very small changes in weight and the owl's flying capability which had a knock-on effect on fecundity. Birds are very efficient animals and must rely on maintaining a delicate weight balance to remain competitively fit (Söhle et al. 2000). Even a small change in a bird's weight loading can cause large knock-on effects on things like flight efficiency but biotelemetry devices can also cause negative effects on much larger, more robust animals. Seals with bio-logging type tags have an average 12% increase in their drag coefficient (Hazekamp et al. 2009) across different types of tags (with and without an additional satellite transmitter) making their swimming processes much more inefficient in comparison to a non-tagged seal.

When the tag itself does not affect the animal directly there are still other concerns. The process of fitting a biotelemetry device can mechanically damage an animal, causing capture myopathy in wild mammals (Abbott et al. 2010). The negative effects of tags can be behavioural as well as physiological. Telemetry packages wrapped in black protective coverings received significantly fewer pecks from Adelie penguins than yellow, blue or white packages (Wilson et al. 1990). A tagged animal is likely aware of the device fitted to it and may invest energy in its investigation, particularly when it is brightly coloured, smells odd or is otherwise noticeable. Such problems are not inherent within all telemetry systems. Meerkats

are unaffected by radiocollars (Golabek et al. 2008) despite being social, curious animals, relatively small and sensitive to weight changes. Biotelemetry devices are still successfully used within ecological studies with little to no effect on the subject animals. It is worth noting, however, that remote sensors are not a silver bullet to protect against observer effects and their affects must be considered fully before use.

Another issue facing biotelemetry, one which is not often reported in studies (Hawkins 2004; Ropert-Coudert & Wilson 2004), is that biotelemetry systems are subject to failure as much as any electronic device. As studies do not report this, it is difficult to know how often this occurs and whether it is improving, however both device failure e.g. the device ceasing to work as expected (Kelly et al. 2010) and fit failure, e.g. unexpected device loss (Kelly et al. 2008) are possible. Additionally, biologgers can also be inaccurate in the data they record, either through device failure or misinterpretation of the data through inadequate algorithms or inadequate sensor design or both (Ropert-Coudert & Wilson 2004). In their review, Ropert-Coudert & Wilson (2004) illustrate how the data recorded by biologgers may be more subjective than initially assumed. Issues with sensitivity and specificity, such as reducing the instances of a device recording a false positive or false negative, are well known and many biotelemetry studies report these and provide algorithms to remove them from datasets (Champion et al. 1997; Tolkamp et al. 2010; Nielsen et al. 2010). Issues with sampling frequency are less well understood. Sampling frequency must be considered in comparison to the frequency and duration of the behaviour of interest (Ropert-Coudert & Wilson 2004). For example, in measuring

play behaviour in calves with accelerometers, Rushen & De Passillé (2012) used a device that sampled at a rate of 33Hz (i.e. 33 times per second) for behaviours which lasted less than 1.5s, e.g. jumps and kicks. This sample rate was sensitive enough to detect the short jumps and kicks of interest. Ropert-Coudert & Wilson (2004) highlight the importance of calibrating any device to sample with a frequency relevant to the behaviour being displayed, but also the importance of detailing such calibrations so studies can be properly replicated. It is important to state all subjective steps taken at the data-analysis stage of studies using biotelemetry. Altmann (2003) said that ethology '*will never be a field that is tool-driven, but we must become a field that is tool-enabled if we are to answer many of the central questions of behavioural biology*'; with these limitations in mind, remote sensors are a powerful tool for the ethologist that enable new areas of research.

1.6 The IceTag activity monitoring system

This project is a BBSRC CASE partnership with IceRobotics Ltd. (South Queensferry, UK) and so the main device utilised in the project was the IceTag®. The IceTag is a tri-axial accelerometer based activity monitor and is a commercially available research device. An accelerometer measures proper acceleration, i.e. the acceleration the device receives proportional to freefall. In layman's terms, tri-axial accelerometer activity monitors work as a glorified pedometer and allow the ethologist to monitor the animal's behaviour remotely. Accelerometers are used in many everyday devices. A smart-phone which can rotate its screen when flipped on its side is detecting this state change by an accelerometer. They are also utilised

'behind the scenes' in many industries, measuring vibrations in structures such as compressors, cooling towers, bridges and roads, etc. It is this widespread usage which has been the driving force for their mass production, and thus reducing their cost to allow for use in a wider range of applications (A. White, pers comm.). It is now possible for ethologists to use tri-axial accelerometer devices to monitor some aspects of animal activity and this is the kind of technology the IceTag is based on.

For the rest of this thesis, I will talk about 'lying' and 'standing' behaviour as recorded by the tags, but a more accurate way of saying this would be 'time the tag accords to lying' and 'time the tag accords to standing'. IceRobotics provide software which interprets the raw accelerometer data from the three axes to judge how the device was orientated. The software assumes the tag is positioned on the leg and so the pitch, yaw and roll of the device are equivalent to the pitch, yaw and roll of the limb at the point of attachment. The software applies a series of algorithms which are proprietary to IceRobotics to interpret any given combination of pitch, roll and yaw as 'standing' or 'lying' for the animal. Standing, in this instance, is also more accurately described as 'time spent upright' as no distinction is made between standing stationary and being in locomotion. IceTags also record MotionIndex™, a proprietary measure to IceRobotics which is a sum of the absolute value of the acceleration at each sample point (corrected for gravity offset) for a given period. Step-count, another measure, is related to MotionIndex. The algorithm for calculating step-count is based on MotionIndex, but step-count is only calculated when the tag is classed as being in a standing position by the IceTag software. This

is because a cow cannot take a step when she is lying down. MotionIndex is recorded continuously, however, and can detect activity of the cow while lying, for example if she rolls, although this is only expressed in an increase in MotionIndex, not interpreted as a behavioural state. IceTags attach between the hock and fetlock joints, usually on a hind leg (see **Figure 1.1**), and so the acceleration summed by MotionIndex is the acceleration the device records at that point. In order to move a limb, the animal must expend energy and so MotionIndex is closely related to the energy expended by that limb and thus the whole animal.

Figure 1.1 *Image of IceTag Pro attached to a beef steer between the hock and fetlock joints*.

IceTags have been thoroughly tested and developed over the past few years, proving to be both specific (proportion of states which were false) and sensitive (proportion of states which were true) in their data recording. A total misclassification rate of 1.7% was recorded by Nielsen et al. (2010). Over a three day period they observed 177 dairy cows and took the last manual observation of lying/standing prior to the removal and reading of the tags. The last observed position of the cow matched the tag's prediction of lying or standing at the same time point, with only two manual observations scoring a cow as lying when the tag data suggested standing and one case of the opposite. This study was manipulative and prompted lying, standing and walking bouts for comparison with tag data. Other studies have compared tag data to manual observations with spontaneous cattle behaviour over longer periods. Initially, large numbers of short duration (<4 minutes) lying bouts were being recorded in studies utilising IceTags. The disproportionately high number of short duration lying bouts was a cause for concern and so Tolkamp et al. (2010) recorded 59,308 lying bouts across three experiments and 73 cows. To form criteria for eliminating false lying bouts they investigated lying durations through video observations and used log-survivorship plots to indicate how frequent bouts were. A minimum lying bout duration of 4 minutes was established. Application of this criterion reduced the number of lying bouts by between 62% and 88% across the three experiments, but only reduced the total estimated lying time by between 0.5% and 3.2%. This is because cows do not lie down for very short periods and these short periods described as 'lying' by the IceTag are more likely to be steps or the cow scratching with her tagged leg. With appropriate algorithms misclassification rates are reduced and IceTags can accurately record lying, standing and step count.

The two main types of IceTag are the IceTag Pro and the IceQube®. Both devices are data-loggers and the key differences between them are in their sample rate and how they store data and this is summarised in **Table 1.3**. Note that although the sample rates are measured in Hz, the data granulation, e.g. the smallest interval in which data is reported, is in minutes for both devices. Therefore each minute block reported by the Pro has 76 sample points, whereas the fifteen minute block reported by the Qube has 3600 sample points. IceTag Analyser reports what percentage (or time duration) of those blocks was spent in lying or standing and a summed MotionIndex. The lower sample rate and coarser granulation of data in the Qube makes it less suitable detecting short, intense bursts of activity and more suited for summarising data over long periods of time. While the Qube can store data for 60 days like the Pros, it can also be set to store data for up to 3 days and transmit this data regularly to a CattleGrid system which stores data for the lifespan of the device. This can be up to five years depending on data storage (IceRobotics 2010). This enables longitudinal research, particularly in dairy systems where a transceiver in the milking parlour will download data multiple times per day. If a researcher was interested in short episodes of activity, such as number of steps over a specific five minute interval, they would wish to use an IceTag Pro, whereas if a researcher was interested in long term recording of activity patterns and was more interested in daily averages, the IceQube would be sufficient. The two tags have very similar sensitivity and specificity results with a 100% correlation between them for lying and standing times and 98% correlation for MotionIndex (IceRobotics 2010). The lower value for

MotionIndex occurs because in IceQubes MotionIndex is summed over the 15 minute interval.

Table 1.3 *Design specifications of the IceTag® Pro and IceQube® data-loggers*

Design Specifications	IceTag Pro Original	IceTag Pro	IceQube
Sampling Rate	16 Hz	16 Hz	4 Hz
Dimensions (mm)	95.0 x 85.0 x 31.5mm	65 x 60 x 30 mm	65 x 60 x 30 mm
Approximate Weight (g)	170g	95g	95g
Data Granulation	1 minute intervals	1 minute intervals	15 minute intervals
Attachment Method	Velcro Strap	Adjustable plastic housing strap	Adjustable plastic housing strap
Data Storage Capacity	On board storage for up to 60 days	On board storage for up to 60 days	On board storage for up to 60 days or for up to 3 days when paired with farm download system, enabling continuous recording for lifespan of tag.

By using a commercial, pre-packaged system such as the IceTag, this project accepts the assumptions inherent within IceTag software, i.e. that a cow is standing when the tag is upright, unless stated otherwise. While accelerometers are growing ever more popular among ethologists, there remains a problem with standardising the methodologies between studies when every researcher uses different 'homebrew' systems for understanding raw accelerometer data (Gao et al. 2013). With an 'off the shelf' IceTag system, any researcher should be able to recreate the work undertaken in this project. However, as these systems may leave the market or be updated we are reliant upon the IceTags being not too dissimilar from custom made systems. No

studies have shown evidence to substantiate this concern, but it is a worthwhile point to raise.

1.7 Conclusions

In efforts to improve welfare and production, animal scientists are exploring individual variation between animals to understand how cattle can adapt to modern husbandry systems. Traits such as the animal's fearfulness or sociability can affect its ability to adapt to its home environment, affecting how the animal responds to potential stressors. Despite this, there is little information as to how personality traits are expressed in the animal's home pen environment. As biotelemetry systems become widely adopted among farmers, databases will form which include vast amounts of activity related data. Before it is possible to utilise this data in welfare assessment, we must understand how activity recorded by activity monitors relates to the personality traits driving behavioural variation in individuals.

1.8 Aims of thesis

The aim of this project was to investigate the possibility of characterising personality and personality traits in cattle through novel applications of the IceTags biotelemetry system. This involved two aspects. First, the physical capabilities of IceTags had to be assessed, with an understanding of what recorded variables could be affected by personality differences. Secondly, this project aimed to assess what behavioural variation might be expressed in activity. Specifically, the aims were:

1. To gain an understanding of the terms ‘personality’ and ‘temperament’ when used in the context of animal behaviour to ensure the terminology is used consistently within the project.

2. To evaluate whether fitting IceTags to cattle could affect their behaviour and so would limit the application of tags in future behavioural studies.

3. To investigate how variation in behaviours displayed in short duration tests of temperament and personality relate to activity behaviours recorded in the home pen using IceTags over a longer duration of time. This will involve:

    a. Determining whether the results of short-term handling tests (often referred to as proxy measures of temperament) correlate with home pen activity.

    b. Determining whether the results of Principle Component Analyses, based on multiple behaviours observed in short-term fear and sociability tests, relate to home pen activity.

Chapter Two

Structuring Consistent Behavioural Variation in Animal Populations: The Difference Between Temperament, Personality and Behavioural Syndromes[1]

[1] In this chapter I was responsible for the review of the literature and the write-up. My supervisors discussed with me the ideas presented throughout. Some of the preliminary work of this review was presented at the ISAE regional conference as **MacKay, Jill R. D**. and Haskell, Marie J. (2012) *A short review of the terms 'individual differences', 'personality', 'temperament' and 'behavioural syndrome' in animal literature, and suggestions as to how to maintain uniformity across disciplines.* Proceedings of the International Society for Applied Ethology UK Regional Conference (Harper Adams). Feedback from this oral presentation contributed to further work.

2.1 Introduction

In the natural world, individuals within a population of animals may consistently vary from one another in their behaviour. This can occur for many reasons, such as age or sex and has historically been viewed as an irritation in animal studies, necessitating many replicates within a study to ensure effects observed are not characteristic of one individual only (Slater 1981). Ethologists find ways to partition off this variation. Some of it can be explained through factors such as age, sex, reproductive status, etc. Honeybees, like many invertebrate groups, have social castes with each caste performing a specific behavioural role for the colony. Yet individuals display behavioural responses that differ from other individuals within the caste (Wilson 1975). Beyond the levels of age, sex, caste, etc. we find behavioural variation displayed in the individual that cannot be wholly explained by other factors. We can apply labels that attempt to characterise the nature of this variation such as: 'fearfulness', 'boldness', 'shyness', 'sociability, 'dominance', 'aggression', 'neuroticism', 'agreeableness', 'openness', 'hostility' and 'stability'. This particular list of labels was collated by a review of the topic 'personality dimensions' in animals by Gosling & John (1999).

What does 'agreeableness' or 'stability' mean? If I describe an animal as 'female' I convey information about the kinds of behaviours I expect that animal will show. I do not expect a female bird of paradise to create a bower, puff up her feathers and dance for a mate. If I go on to describe her as 'agreeable', what am I implying about the behaviours I expect the bird to show? Would I expect to be able to handle this bird easily, to find her in close proximity to an undesirable stimulus, or would she

attack me repeatedly and always choose the most peaceful area in which to make her nest? 'Agreeableness' is a vague descriptor and arguably may not be relevant or informative of behavioural variation within the species and so it may not be the most suitable label for characterising variation in this hypothetical bird, despite the label's wide usage elsewhere (Uher 2008). In science we wish to understand or characterise behavioural variation on many levels, including at the individual level. Even the most vague and undefined of these descriptive labels allow for some prediction of the individual's behaviours.

The labels discussed above are not directly quantifiable. There is no unit of fear, openness or neuroticism, but through careful interpretation of behaviour (Graham et al. 2003; Carter et al. 2012a) we are able to measure and record behaviours related to each descriptive label. This phenomenon of consistent individual behavioural variation is complex with many concepts surrounding it. In an attempt to make a simple analogy, imagine that we have discovered a new species of animal. Think of an animal's response to an environmental challenge as being similar to dice being thrown. With no prior knowledge of the animal's inner state, e.g. when it last fed, its prior experiences or the current environment, then to the observer, the behaviour the animal shows after exposure to the challenge could appear as random as a thrown die. If, on repeated observation of the dice, we find we can characterise the behaviour as non-random, we establish that the individual die are loaded. This would be similar to applying the descriptive label of 'fearfulness' to an animal after repeated observations of its behaviour To an observer, a 'fearful' animal is playing with loaded dice. Fearful behaviours would be more often prioritised by the animal, so

one side of the dice comes up more often than is statistically probable. As the observer gains more information about what side of the die is loaded, the observer is more able to predict what will come up. We cannot take the die apart to get an exact measure of the weighted side, but we understand that the pattern of numbers which come up is not random. The weighting is not quantifiable, but the outcome of the weighting in the patterns observed is, just as we cannot quantify the level of 'fear' an animal's nervous system, but we can quantify the behaviours the animal expresses. This is the issue of measuring 'fearfulness'. As this is a new 'species' of dice animals, we could not simply pick up one dice and throw it once. It would be necessary to throw the die several times to realise we were observing a non-random pattern of behaviour. It would also be necessary to observe several dice to understand if there was variation in these non-random patterns. With only one individual, we do not know if all dice are loaded in the same way or if for different individuals the dice are loaded differently, and whether this difference in loadings manifests on the same side or differently on all sides. It may be that all dice are loaded on a specific side but some are weighted more heavily than others. Or it may be that all dice are loaded on different sides to different extents. Only by observing multiple individuals over time can we characterise how the different loadings manifest in what sides are shown. When we have established the patterns within the population, we might be more comfortable with stating that a loading exists when throwing a single die once, and this is related to the repeatability of the behaviour.

There are two important concepts in the study of this behavioural variation in animals. The first is that individuals may *consistently vary from one another* in the

way they respond to stimuli or challenges and that the variation is repeatable across time and possesses a heritable component (Wilson 1998; Herborn et al. 2010; Stamps & Groothuis 2010). The second is that different traits may be linked *across different contexts* (Sih et al. 2004; Bell 2005; Herczeg & Garamszegi 2011). One pioneering study in this area found that sticklebacks which are more aggressive to intruders than others within the population during the breeding seasons, tend to be more aggressive to predators outwith breeding seasons (Huntingford 1976). This study established that individual sticklebacks varied consistently in the levels of aggression they showed under standard conditions, but also established that these differences were maintained across situations and time.

2.1.1 Terminology surrounding consistent individual behavioural variation

This phenomenon has been studied across many different disciplines of biology for many years and as a result there are many different approaches. This is echoed in the wide array of terminology used to discuss and quantify the phenomenon. Several words such 'context', 'trait' and 'repeatability' are given specific meanings when discussed in relation to consistent individual behavioural variation (a summary of how these are defined in recent reviews is given in **Table 2.1**). Note that Uher (2008, 2011) gives two different definitions for 'trait' within biology and psychology. The psychology definition is more similar to that of a 'personality dimension', i.e. something that is not immediately apparent within the population, demonstrated by her description of a trait being a 'latent dimension'. She notes that in biology, traits are empirical measures of an individual but that this does not refer to the theoretical structure of behavioural variation that would be inferred from this. It is also worth

referring to Sih et al.'s (2004) distinction between a context (as a functional behaviour category) and a situation (a set of environmental conditions). As this chapter will go on to demonstrate, Sih et al. (2004) is a highly influential paper, but the differences in the way he defined 'context' and 'situation' are not always upheld in the literature. If context is used in a manner to suggest a functional behaviour category it is usually stated as 'feeding context', 'parental care context' etc.

Table 2.1 *Commonly used terms in behavioural variation*

Term	Meaning	Reference
Context	A functional behavioural category such as 'feeding', 'mating', 'parental care', 'contests', etc.	(Sih et al. 2004)
Situation	A given set of environmental conditions at a certain point in time, e.g. high predator risk	(Sih et al. 2004)
Trait (psychology)	Latent dimensional variables on the population level that have the same meaning in all individuals which permits comparing the relative positions of individuals to one another on these trait dimensions	(Uher 2008)
Trait (biology)	Any empirical measure obtained from an individual, but not the theoretical concepts that are inferred from such measures.	(Uher 2011)
Repeatability	Standardised measure of the differentiation in average phenotype across individuals, defined as the proportion of phenotypic variance explained by differences between individuals	(Dingemanse et al. 2010b)

2.1.1.1 Repeatability

Table 2.1 refers to repeatability as a measure of what proportion of the variation in the average phenotype can be explained by the differences between the individuals. This definition was taken from Dingemanse et al. (2010b). As this chapter has so far discussed *consistent* individual behavioural variation, and repeatability is a popular measure of consistency, it is worth briefly outlining what is meant by the term

repeatability. Most studies (Bell et al. 2009a) utilise the variance method calculated as in Lessells & Boag (1987) to estimate repeatability where:

$$Repeatability = \frac{variance\ among\ individuals}{variance\ within\ individuals + variance\ among\ individuals}$$

Bell et al. (2009a) conducted a thorough meta-analysis of repeatability in behavioural studies in order to answer some fundamental questions about repeatability, such as does it vary among age groups and does it decrease with the interval between observations? The main findings of Bell et al. (2009a) were that the average repeatability of a behavioural trait was 0.37 and that weighted for effect size across all estimates, repeatability was significantly greater than zero. As they put it, the data overwhelmingly supports the hypothesis that behaviour can be repeatable. Repeatability does tend to be higher for behaviours measured closer together in time, but they found it more difficult to draw conclusions as to whether repeatability varied between age groups, but concluded that overall they found no evidence of a difference in repeatability between juveniles and adults, except in some ectotherms. They concluded that the research strongly supported the idea that individual behavioural differences were consistent with time.

We are interested in investigating why an animal shows a consistently non-random pattern of behaviour, but there are many biologically valid reasons why an animal's response to a situation might change over time, meaning that repeatability would not exist in the trait. Animals can habituate to a stimulus, learn a different response, or the trait itself may not be a repeatable trait. Dingemanse et al. (2010b) have produced an excellent review reconciling the plasticity of animal behaviour with the aspects of

consistency that interest us when investigating consistent individual behavioural variation. Repeatability may not exist where habituation, the reduction in the size of a behavioural response, has occurred. Habituation is a problem in experiments relying on novel stimuli (Erhard et al. 2006). What may be startling at first exposure becomes routine after successive exposures. Carter et al. (2012b) detail the difficulty of testing for the same stimulus, such as novelty, as novelty is reliant upon the first time the animal is presented with the stimulus, but different kinds of novel stimulus may not be comparably alarming.

The animal can also learn from different experiences associated with stimulus, and learning can change their response. How an individual may learn from prior experience can be very complex. Rapid assays of labile behavioural traits on Ward's damselfish (*Pomacentrus wardi*) show a range of responses from habituation, to sensitisation and even no change (Biro 2012). From this, Biro suggested that laboratory or unusual environment work may need to be repeated more often than is already done in order to safely assume that rank order differences across individuals is maintained. This recommendation was criticised by Edwards et al. (2013) for the very reason mentioned previously, that the prior experience of the fish changed their perception of the stimulus they were responding to, meaning it was no longer comparable between individuals.

Despite the issue of behavioural plasticity, the repeatability of behavioural traits is an extremely important part of the study of consistent individual behavioural variation. There are clearly limitations to repeatability, and behavioural plasticity is a topic

worthy of several chapters within its own right but for the purposes of this framework we will assume that the hypothetical traits being referenced have been found to be repeatable with all appropriate measures taken to ensure this.

2.1.2 Terminology referring to consistent individual behavioural variation

From the reviews cited in **Table 2.1** it is possible to collate some of the phrases which exist to provide a label for the phenomenon at large. These are 'consistent individual behavioural variation', 'individual behavioural characteristics', 'individual differences', 'individual distinctiveness', 'behavioural types', 'behavioural phenotypes', 'individual predisposition', 'individual disposition', 'behavioural styles', 'behavioural strategy', 'behavioural profiles', 'behavioural characteristics', 'coping styles', 'coping strategies', 'reactivity', 'responsiveness', 'handling temperament', 'risk taking', 'temperament', 'personality', 'personality dimension', 'personality trait', 'temperament trait', 'personality factor', 'animal personality', 'character', 'behavioural syndromes' and 'distinctiveness' to name but a few. In his cross-disciplinary review Gosling (2001) highlighted how this variety of terminology has negatively impacted the study of the phenomenon in animals:

> "*A considerable number of publications on animal personality exist, but they are dispersed across a wide range of fields and are hard to find".*

And this is echoed almost a decade later by Archard & Braithwaite in their 2010 review:

> *"The impact that such [individual differences] have on behaviour has only recently become of interest for behavioural and evolutionary ecologists. Two main reasons for this have been the lack of consistent*

terminology (e.g. 'personality', 'temperament', 'coping styles' and 'behavioural syndrome' are all found in the literature) and the lack of ecological and evolutionary framework for temperament studies."

This lament has been oft-repeated in several reviews of animal behaviour (Phillips & Peck 2007; Brydges et al. 2008; Turner et al. 2011a; Titulaer et al. 2012). Regardless of the confusion in definitions, these reviews share the general consensus that behavioural consistency exists in individuals, can affect the animal's health, reproductive success, survival, welfare and productivity, and through the animal's interactions affect the life of their conspecifics and nonconspecifics, e.g. human-animal interactions.

Archard & Braithwaite (2010) declared that an ecological and evolutionary framework was needed for studies concerning consistent individual behavioural variation in animal species. This chapter will demonstrate that such a framework already exists within commonly used terminology, only comprehended when behavioural variation is considered within the concepts of between individual and between context behavioural variation. This thesis will then use this proposed framework to investigate the relationship between personality in cattle and activity that can be measured via biotelemetry systems.

2.2 Refining terminology

With so many different terms to choose from, proposing a framework seems at first a daunting task. It is necessary to produce criteria for refining the terms. Firstly, we are interested in the behavioural variation so we can remove terms which can also refer

to physiological variation. Secondly, we can remove redundant terms by grouping variants under the widest label. Thirdly we will remove terms which do not consider underlying structures of the variation, for example any terms which are used to address stand-alone observations or characterisations of behaviour without referencing other individuals or populations.

Terms such as ‘distinctiveness’ and ‘predisposition’ do not immediately address behaviour unless phrased as ‘distinctiveness in individual behaviours’ or similar, whereas terms like ‘temperament’ are clearly referencing the phenomenon of interest. Some of the terms such as ‘character’, ‘coping styles’ and ‘individual differences’ also incorporate physiological variation between individuals as well as behavioural (Uher 2011). Indeed, in the comprehensive review of ‘coping styles’ by Koolhaas et al. (1999) they highlight that inherent within the definition of ‘coping styles’ is the measurement of physiological parameters and note that many studies have failed to include these parameters, weakening the study of ‘coping styles’. Therefore any phrase which does not inherently imply behavioural variation is eliminated in **Table 2.2**.

Some of the terms in section 2.1.2 can be amalgamated. For example, ‘personality traits’ and ‘personality dimensions’ clearly have some bearing on ‘personality’ and this is further indicated in **Table 2.2**. Some of the phrases can be difficult to amalgamate, such as those containing the term ‘behavioural’. The number of returns which can be found when searching for each term in the ‘topic’ field of Web of Science is given as an indication of how it has been used from 1970-2012 in the

scientific literature. As a comparison of human orientated sciences versus animal sciences, the number of articles found in the fields of evolutionary biology, ecology, veterinary sciences and zoology are also given. ‘Behavioural strategies’ and ‘behavioural phenotypes’ have 366 and 119 returns in these fields respectively. It should be noted that ‘behavioural syndromes’ is a relatively new term but has 258 returns in these fields. ‘Behavioural syndromes’ has been a topic of much discussion since Sih et al.'s (2004) influential paper, which according to Web of Science has had more than 570 citations as of January 2013. In comparison, only ‘behavioural strategies’ can compete with the level of interest ‘behavioural syndromes’ receives and so ‘behavioural strategies’ and ‘behavioural syndromes’ may be retained.

Lastly we investigate which phrases refer to an underlying structure of variation. The term ‘risk taking’ is a stand-alone observation of risk and so does not refer to an overall structure of variation (although we might imagine that ‘risk taking’ is linked with ‘boldness’, we would suggest that it is the level of ‘boldness’ which drives variation in ‘risk taking’ and so ‘risk taking’ itself does not directly link to the structure of variation called ‘boldness’). ‘Consistent individual behavioural variation’ simply refers to the variation between individuals and does not necessarily imply an underlying structure. ‘Personality’ however, being based on ‘personality traits’ does imply structure, as does ‘temperament’ with ‘temperament traits’ therefore we may keep the latter two terms. ‘Behavioural syndromes’ is used at the individual and population level, however ‘behavioural strategies’ is used to discuss differences in behaviour across species, which is too broad a label for our purposes. A difference in species would be a solid biological basis for expecting behavioural

variation between two individuals and so ‘behavioural strategies’ in its common usage is not suitable for our purposes.

This criteria has left us with three terms are commonly used and which specifically reference behavioural variation between individuals. ‘Behavioural syndromes’, ‘personality’ and ‘temperament’ will therefore be used in this chapter. That is not to say that the other terms have no value, indeed ‘consistent individual behavioural variation’ is a phrase that will be used repeatedly in this chapter, but that for the purposes of formalising a framework, these three terms are best suited.

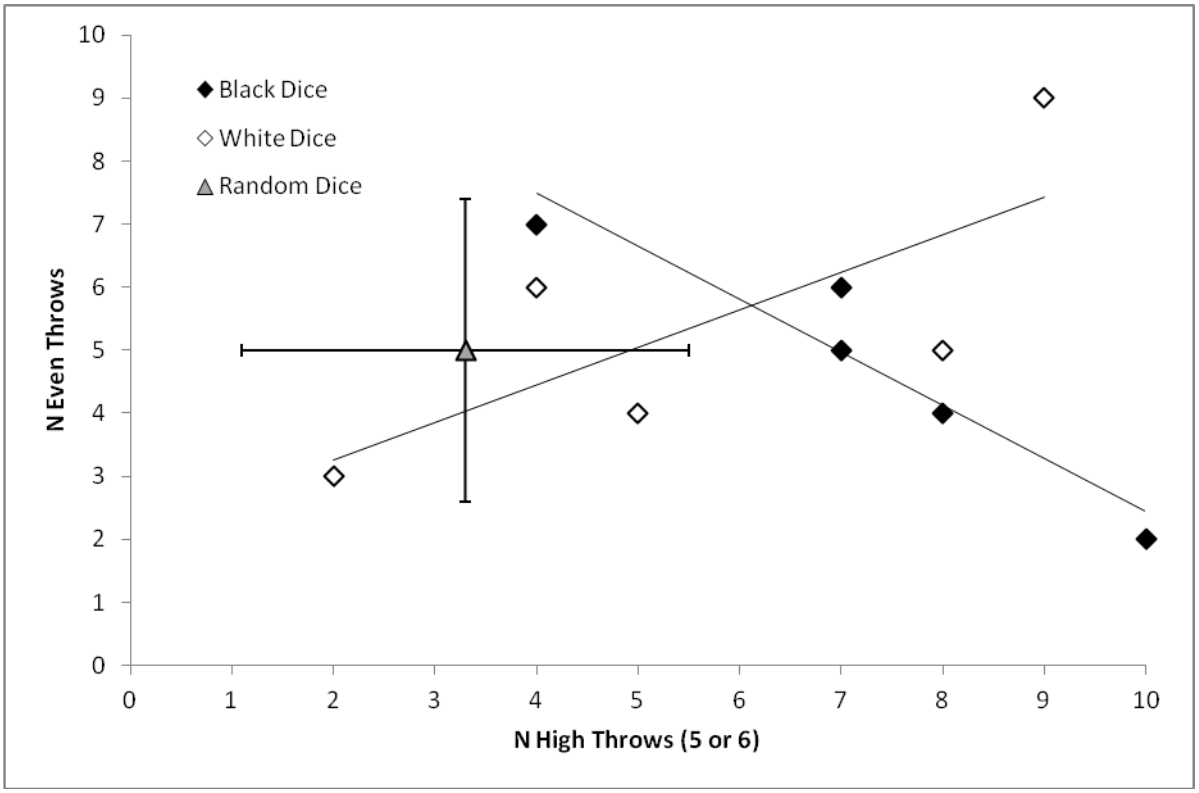
Table 2.2 *Refining terminology based on three criteria. Does the term discuss behavioural variation only? Can the term be folded into another term and if not, how frequently is it used? Does the term reference the structure of variation we are interested in, between individuals and between populations? Number of returns in total and number of returns in the Web of Science categories 'Ecology', 'Evolutionary Biology', 'Zoology' and 'Veterinary Sciences' for comparison, data from 1970-2012, correct as of January 2013. Shaded cells denote when term dropped from consideration (see text).*

Term	Discusses behavioural variation only?	Redundancy? If confusion, N Returns in Web of Science Given		References structures of variation?
		N Returns	N Returns in fields of evolutionary biology, ecology, veterinary sciences or zoology	
Behavioural phenotypes	Yes	2404	119	
Behavioural profiles	Yes	1355	93	
Behavioural strategy/strategies	Yes	1977	366	No
Behavioural styles	Yes	99	8	
Behavioural types	Yes	169	57	
Behavioural Syndromes	Yes	829	258	Yes
Character	No			
Coping Styles/Strategies	No			
Distinctiveness	No			
Consistent Individual Behavioural Variation	Yes	No		No
Individual Behavioural Characteristics	Yes	Variant of 'Consistent Individual Behavioural Variation'		
Individual Differences	No			
Individual disposition	No			
Individual Distinctiveness	No			
Individual predisposition	No			
Personality	Yes	No		Yes
Personality Dimension	Yes	Variant of 'personality'		
Personality factor	Yes	Variant of 'personality'		
Personality Trait	Yes	Variant of 'personality'		
Animal Personality	Yes	Variant of 'personality'		
Reactivity	No			
Responsiveness	No			
Risk Taking	Yes	No		No
Temperament	Yes	No		Yes
Handling Temperament	Yes	Variant of 'temperament'		

2.3 Structures of behavioural variation, the use of reference points between entities and contexts

Before discussing what each term means and how to fit them to a framework it is necessary to discuss the nature of the behavioural variation that can be observed and measured and how we attempt to characterise this. Earlier, in discussing the Huntingford (1976) study, two important concepts were raised in the study of consistent individual behavioural variation: consistent variation between individuals and consistent variation across contexts. What is the behavioural variation we are interested in characterising? To explain this, it is time to return to the dice analogy. Say we have ten dice and what we truly want to know is what makes these dice show non-random patterns. However, we are not able to take the dice apart to weigh these loadings, so instead we will observe their behaviour over time. After some preliminary observations, we decide there are two traits (i.e. latent dimensions within the populations which allow for comparison of individuals) that we are interested in. Are the dice weighted for high numbers or are the dice weighted for even numbers? Without weighing the dice, we decide to quantify this using the proportion of times a thrown dice will show an even numbered side and proportion of times a thrown dice will show the high numbered sides 5 or 6. As we aware that repeatability is important, we throw each dice ten times and record proportions of traits of interest. We are also aware that our ten dice come from two different sets, five of our dice are black and five of our dice are white, so we will record this also. For comparison, for a random population we would expect approximately half of the throws to come up even and one third of the throws to come up high. The results of this hypothetical experiment are shown in **Figure 2.1**

Figure 2.1 *Hypothetical experiment recording two behavioural traits (even loadings and high loadings) in two populations of dice (black dice and white dice). Random chance would expect 1/2 of throws to be even and 1/3 of throws to be high. Standard error bars of random throws estimated by random number generator in Excel.*



In this example, there are several interesting structures of behavioural variation. Firstly, as this is a hypothetical world, the two behavioural traits 'even' and 'high' are the only ways the dice may vary. Therefore, the x,y coordinates of each individual point on the graph completely addresses all of the variation present in the individual die (in the real world we would need a seven dimension graph to account for the six possible sides and the additional variable of 'even' but not in our hypothetical experiment). For each individual die, the x,y coordinates are a reasonable predictor of the outcome of the next throw.

Another interesting structure of variation comes from looking at the two populations, the black and the white dice. Within the black dice, it appears that the more high throws a dice records, it is likely to also being throwing fewer even numbers (presumably these high throws are predominantly the 5 side). However, within the white dice, it appears that many high throws are associated with many even throws (presumably these high throws are predominantly the 6 side). This may tie in with repeatability and within individual variation, how often does each individual die show a 5 or 6, or an even side? Additionally, there is considerably more spread within the white dice, whereas the black dice follow the trendline more closely. Here there are two further structures of variation, the relationship between the two traits in each population and the extent to which each population fits that relationship.

Now that we have identified the structures of consistent individual behavioural variation that we are interested in, can we find references to these structures in the literature?

2.4 It all means the same in the end?

The three terms ‘behavioural syndromes’, ‘personality’ and ‘temperament’ each imply something about the structure of the behavioural variation observed. There is some debate, however, on whether all three terms comment on the structure of behavioural variation in the same way. If all three terms are synonymous, it would be easier to simply refer to one term and leave out the others, however some researchers maintain that there is a difference in the meanings of the terms. This section will investigate why there is a tendency to use the terms interchangeably within the

literature and why there are in fact differences in their meanings when considered in terms of the underlying structures of consistent individual behavioural variation.

2.4.1 Why is there confusion?

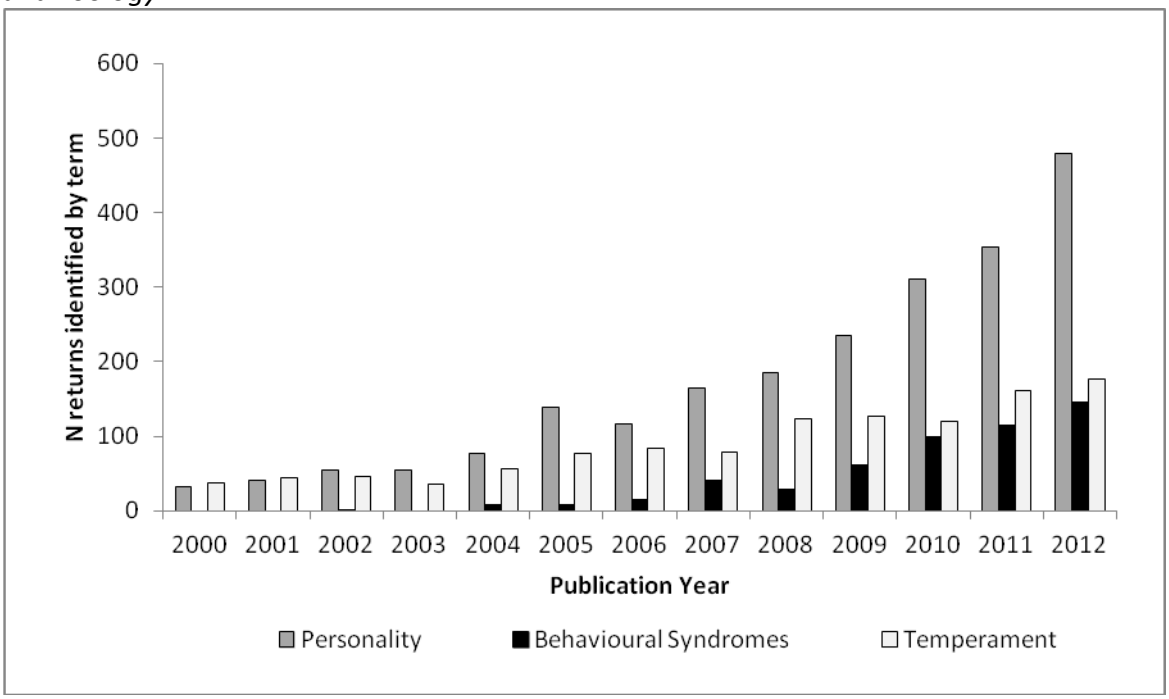
First of all, it is necessary to understand what is meant by the three terms 'behavioural syndromes', 'personality' and 'temperament' as they are currently used in the literature. Web of Science is an important resource for researchers today, enabling the cataloguing and interrogation of vast numbers of scientific articles. Web of Science also tracks the number of times each article has been cited. While a highly cited paper does not necessarily mean that the research in general agrees with it, it does imply that the paper has been widely seen. Throughout this section, several definitions for each term will be given from many different scientific fields. For the sake of reference, **Table 2.3** lists definitions for each of the three terms from the most cited articles in the fields of ecology, evolutionary biology, zoology and veterinary sciences. 'Behavioural syndromes' are referred to most commonly as suites of correlated behaviours across situations or contexts (see note on **Table 2.1** regarding usage of situations and contexts) which exist within populations (Sih et al. 2004; Bell 2007; Dingemanse et al. 2007). 'Behavioural syndromes' are also referred to as existing within individuals (Dingemanse et al. 2010b), which will be investigated later. 'Personality' is most commonly defined as a style of behavioural response to a range of stimuli or situations and refers to individuals (Wilson et al. 1994; Gosling & John 1999; Uher 2008), whereas 'personality dimensions' or 'personality traits' are found in populations and species. 'Temperament' is often referred to as the individual reaction to a challenging situation (Rothbart 2007;

Dugatkin 2009; Uher 2011), again with 'temperament trait' being a little more general and referring to a population.

Table 2.3 *Definitions for 'personality', 'temperament' and 'behavioural syndromes' in the most cited articles returned by a Web of Science search for each topic between the years 1970-2012 in the Web of Science categories agriculture, behavioural sciences, ecology, evolutionary biology, veterinary sciences and zoology. (Exclusively human orientated papers excluded) Citation counts correct as of March 2013*

Term	Definition **(Italicised text denotes reference to between individual or between group variation. Underlined text denotes reference to within or between context variation)**	Source	Citations
Behavioural Syndromes	Recent studies suggest that *populations* and *species* often exhibit behavioural syndromes; that is, suites of correlated behaviours <u>across situations</u>.	(Sih et al. 2004)	593
Personality	[In discussing a shy-bold continuum] Almost everyone who observes animals is impressed by the behavioural differences that exist among individuals, even within the categories of age, sex and size. A few behavioural ecologists have actually studied these differences by systematically exposing individuals to a variety of stimuli and noting their response.	(Wilson et al. 1994)	350
Temperament	Temperament is defined as how an *individual* reacts to <u>novel or challenging situations</u>. This concept originated from child psychology but has recently attracted the attention of ethologists and evolutionary biologists	(Réale et al. 2000)	161

Figure 2.2 *Number of articles per publication year for topic terms 'personality', 'temperament' and 'behavioural syndromes' in Web of Science categories agriculture, behavioural sciences, ecology, evolutionary biology, veterinary sciences and zoology.*



Why then is there confusion? As mentioned previously, 'behavioural syndromes' have been a topic of much discussion since Sih et al.'s (2004) influential paper. **Figure 2.2** shows how the usage of all three terms has increased since the turn of the century and 'behavioural syndromes' has experienced an eighteen-fold rise since 2004, compared to a six-fold rise for 'personality' and three-fold for 'temperament' over the same period. The confusion mostly stems from the rise in interest in consistent individual behavioural variation, as demonstrated in **Figure 2.2**, but also because there have been some misconceptions and a lack of understanding of key points, not only between all three terms, but also within the usage of the terms themselves.

2.4.2. Confusion between all three terms.

Behavioural syndromes are often stated as being analogous to personality (Bell 2007) or animal personality (Van Oers et al. 2005; Sinn et al. 2008; Dingemanse et al. 2010a), or a personality trait (Smith & Blumstein 2007). This is an interesting idea, suggesting that behavioural syndromes and personality are comparable (but importantly, not the same) in certain respects. It is not surprising, nor wrong, that when new terms are introduced into the literature they are accompanied by an existing term, more familiar to the audience, to aid in understanding. It is important to note however that 'personality' and 'animal personality' have been considered two separate things by psychologists and are not analogous themselves, with conceptual misunderstandings giving rise to the idea that the common usage of 'animal personality' is the same as how psychologists use 'personality' (Uher 2011). Uher argued that ethologists use 'animal personality' more similarly to how a psychologist uses the term 'personality trait', i.e. a structure of behavioural variation within a population. Furthermore, a psychologist would certainly not consider a 'personality' equivalent to a 'personality trait' (Pervin 1994). Hofstee (1994) suggested that a personality trait should be considered a 'point of reference' for the study of the individual's personality as a whole, e.g. that traits should be considered as a way of scaling variation within a population. An individual can only be at a high extreme of the scale in comparison to the distribution of individuals along that scale. In practical terms the trait of 'boldness' allows me to declare an animal 'bold' in comparison to the rest of the population, implying there are less 'bold' individuals in the population. The other important limitation on the use of behavioural syndromes, discussed by

Stamps & Groothuis (2010) and in the following section is that behavioural syndromes occur in a set of individuals, not within one individual. Personality traits occur in a set of individuals and are a construct used to describe the range of behavioural variation in a cluster of similar responses (Digman 1990; John & Robins 1994; Uher 2011) e.g. the human Five Factor Model (FFM) of personality attempts to describe the human population across cultural and sociological boundaries using the five dimensions of neuroticism, agreeableness, extraversion, openness and conscientiousness (although this warrants further discussion below). In contrast personality is the sum of those traits in the individual (Chatterjee et al. 1992; Pervin 1994; Rothbart 2007; Uher 2011). So how can a behavioural syndrome be similar to both a personality and a personality trait when these two things are different, and additionally the common definition of behavioural syndromes implies that a behavioural syndrome refers to more than one behavioural trait? The common analogy made between personality or personality traits and behavioural syndromes is very misleading.

At this point we must further discuss the issue hinted at in the previous paragraph. Another source of confusion in the literature is the use of trait theory. That is to say there are dimensions of variation in the behavioural responses shown within a population and all members of the population have a position along these dimensions, whether that be extremely low, extremely high or somewhere in the middle. This has previously been discussed obliquely in the dice example where the weight of the dice could not truly be measured. The FFM is often considered a good example of this, as it is supposed to describe a great deal of behavioural variation

within the human population. Unfortunately, it is not truly universal across the human population, despite frequent assumptions that it is a 'biologically based human universal' (Gurven et al. 2012). The Tsimane forager-horticulturalists in Bolivia are an isolated community studied by Gurven et al. (2012) with extended family clusters and a language which features 'good' and 'bad' judgements stated in various domains and descriptions. The outcome of Tsimane personality tests did not produce a good fit with the FFM, with extraversion, agreeableness and conscientiousness performing best but low internal reliability being found throughout. Furthermore, Spanish-speaking Tsimane did not fit the FFM any better. Gurven et al.'s study highlights the problem of adopting a model too readily without appropriate validation. Hofstee (1994), in criticising the wide adoption of trait theory, posits an excellent rationale for why traits are so popular:

> *"There can be no reasonable doubt that the [Five Factor] model represents a step forward in a domain that was characterized by conceptual and operational anarchy. But it would be equally unreasonable to claim definitive status for it. For example, it is highly unlikely that genetic research will one day pinpoint exactly these five biological determinants of personality."* Hofstee (1994)

Yet in ethology we talk about measuring (temperament) traits (Visser et al. 2001; Van Reenen et al. 2005) and even the inheritance of (temperament) traits (Reverter et al. 2003; Kadel et al. 2006). Is it wise to link the so-called 'big five' personality traits recorded in humans to behaviours recorded in animals (Wilson et al. 1994; Gosling & John 1999; Sinn et al. 2008), especially when this approach is not favoured by all human psychologists? The danger of wrongly interpreting behaviours and forcing

them into certain models of personality are another debate and is discussed by Uher (2011). The need for appropriate rigorous methodology in validating trait testing is discussed very well by Carter et al. (2012b; 2012a). This caution also applies when temperament tests in specific contexts are assumed to be driven by a certain personality trait (Réale et al. 2007; Archard & Braithwaite 2010), at least for psychologists. Here the important concept is that *traits are a model of variation within a population*, not a tangible, easily quantified thing. For example, boldness is a well studied personality trait in many species (Wilson et al. 1994; Gosling & John 1999; Svartberg 2002; Sinn et al. 2008) but there is no unit of boldness. The FFM is a popular and widely known personality model, however Gosling & John (1999) note that in animals, many of the dimensions are contracted or removed. Some researchers have worked on meta-analyses of behavioural studies and suggested that animal behaviours can be explained by two factors, activity-exploration and fear-avoidance (Budaev 1998; Budaev 2000). This is conceptually very similar to two dimension models of temperament proposed by Van Reenen (2012) where behavioural variation is split into two dimensions, the quantitative activity (high/low) and qualitative (active coping/passive coping) dimensions. One could argue that the active/passive models are incorporated into the term 'personality' as two-dimensional models as opposed to the more complex models in human psychology such as the FFM, Belbin's Team-Role Model, etc.

It is important to realise that the Five Factor Model of personality (and, more generally, trait theory as a whole) is only ever intended to be a model for characterising behavioural variation. It is also a very general model, allowing for

extraversion, neuroticism and agreeableness to be found across many species (Gosling & John 1999). Models, and therefore trait theories, are not faultless, nor are they even 'real' quantifiable things. They are simply a way of characterising variation to allow us to compare individuals.

2.4.3. Confusion within the usage of 'behavioural syndromes'

2.4.3.1 Across time versus across contexts

As well as Sih et al's definition given in **Table 2.3** another, slightly different definition of behavioural syndromes is given by Bell (2007) when discussing future directions of research. In that review, Bell says behavioural syndromes occur when individuals behave consistently across time or across situations. Most authors cite the second part of this definition, across contexts, however some researchers have noted the first part of this definition also. In their 2010 review, Stamps & Groothuis considered that:

> *"'Personality' is a term borrowed from psychology, where it refers to underlying behavioural tendencies that differ across individuals, that are consistent within individuals over time, and that affect the behaviour that is expressed in different contexts . . . By contrast, the term 'behavioural syndrome' is currently defined as individual differences in behaviour patterns that are either correlated across time (e.g. aggressiveness of the same set of individuals before* versus *after sexual maturity), or across contexts (e.g. activity of the same set of individuals in their home cages* versus *in an open field). Hence, any behaviour that satisfies the criteria for personality also satisfies the*

criteria for a behavioural syndrome, but the reverse is not the case."

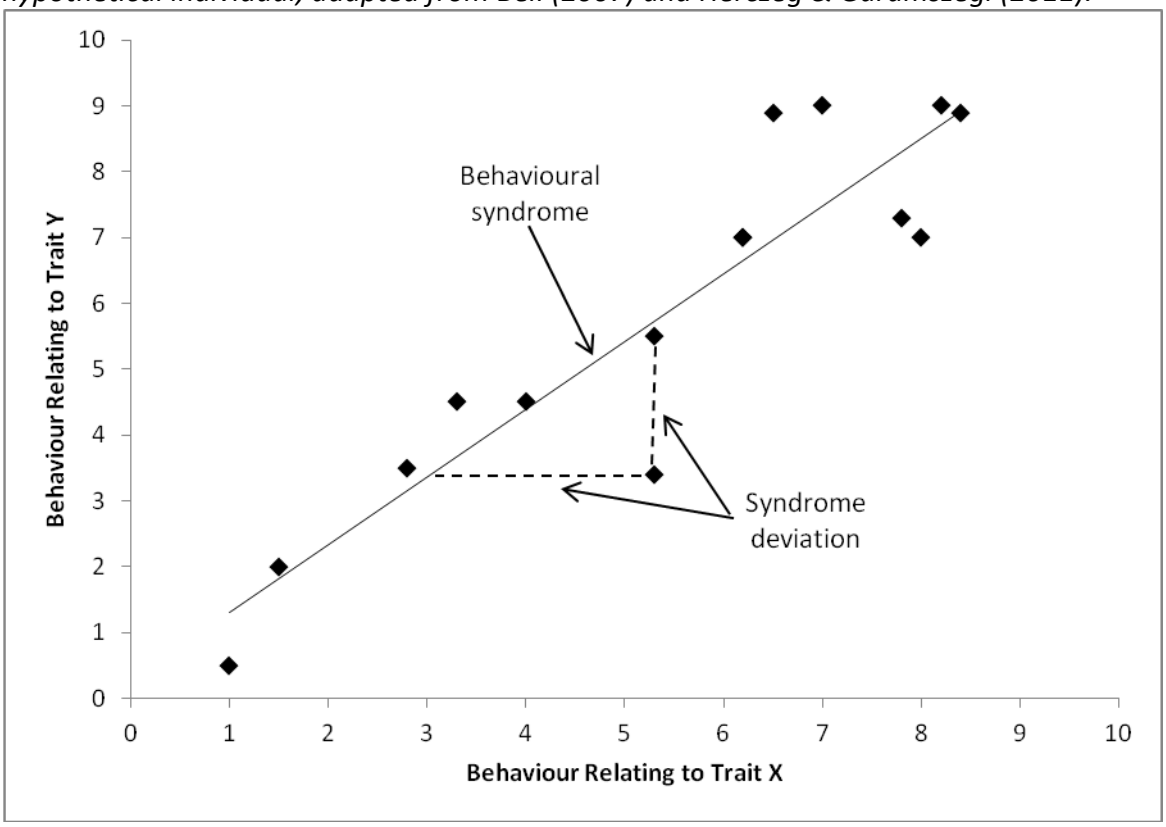
(Stamps & Groothuis 2010)

Note the usage of 'context' in this case to be similar to 'situation' as it would have been defined by Sih et al (2004). This is an interesting statement which implies that personality affects consistency over time and contexts, but that behavioural syndromes may or may not cover all of those aspects. As will be demonstrated in the following discussion of behavioural syndromes, this second definition of 'across time' is less commonly used in recent papers, perhaps because, as Stamps & Groothuis suggest, it is already incorporated in the term of personality. As we are interested in consistent individual behavioural variation, some element of repeatability through time is inherent in any definition and it seems odd to create a definition that does not account for it. The extent to which behavioural variation is repeatable or plastic through time is the subject of many reviews in itself (Bell et al. 2009a; Biro 2012; Edwards et al. 2013) and so this framework will concentrate on the first definition of a relationship across contexts.

Bell (2007) represents a behavioural syndrome diagrammatically and this definition (adapted in **Figure 2.3)** has been used by many researchers including in a set of recent papers debating how behavioural syndromes should be considered statistically and in meta-analyses (Dingemanse et al. 2010a; Herczeg & Garamszegi 2011; Garamszegi & Herczeg 2012). Here it is clear that in this definition the behavioural syndrome is a function of the relationship of two different traits within the population and can only be seen in the set of individuals. Examining one individual's point on this figure could not tell you about the behavioural syndrome itself whereas

Dingemanse et al (2010a) note that an individual can exhibit a behavioural syndrome. This may be more related to the definition incorporating consistency across time which, as previously mentioned, is less useful when discussing consistent individual behavioural variation.

Figure 2.3 *Schematic representation of a behavioural syndrome (correlation) between behavioural traits expressed by a set of individuals. Each point represents a hypothetical individual, adapted from Bell (2007) and Herczeg & Garamszegi (2011).*



2.4.3.2 Behavioural syndromes at the level of the individual

Another source of confusion is the tendency to consider behavioural syndromes as a presence/absence trait within a population. Herczeg & Garamszegi (2011, Haramszegi & Herczeg 2012) argue that studies of behavioural syndromes often seek to address the possibility of them being a result of a constraint hypothesis, i.e. that

two behavioural traits are linked by evolution, such as a genetic link (Van Oers et al. 2005) and thus are constrained from varying independently. Alternatively, the evolution of behavioural syndromes is adaptive, that linking two traits has a positive impact on survival (Bell 2005). In both cases, Herczeg & Garamszegi are critical of simply using the existence of a behavioural syndrome within a population to 'prove' either the constraint or adaptive hypotheses. They argue that by treating behavioural syndromes as either 'present' or 'not present' we lose ability to address these two hypotheses (Herczeg & Garamszegi 2011; Garamszegi & Herczeg 2012). Herczeg & Garamszegi argue that behavioural syndromes should be considered as a continuous structure of variation which individuals may deviate from. They introduce the term 'syndrome deviation' as a measure of how the individual differs from what the behavioural syndrome would predict. For example, if the behavioural syndrome predicts that aggression and boldness have a correlation of r=1, but individual A deviates from this, the distance of its own behavioural responses from the predicted syndrome is the syndrome deviation. By treating the behavioural syndrome as a continuous trait, Herczeg & Garamszegi argue they can more fully understand the fitness advantages of a syndrome at the individual level, allowing for greater testing of different hypotheses.

2.4.4 Confusion within the usage of 'personality' and 'temperament'

When giving an introduction to the idea of 'personality' in animals Gosling (2001) grouped together 'personality' and 'temperament' results, in part because the research had already done so, and partly because he noted that 'temperament' appeared to be used in substitution for personality due to fears of anthropomorphism.

There is no doubt that accusations of anthropomorphism have contributed a great deal to the conflagration between temperament and personality in the study of animal behaviour. This idea of 'personality' being 'of a person', i.e. human, was widely accepted in science several decades ago with one human psychology review saying:

> *"When we apply the notion of personality in order to characterize animal behaviour we tend to use it parenthetically. This is not so with temperament, a concept applied to characterize both human and animal populations."* (Strelau 1987)

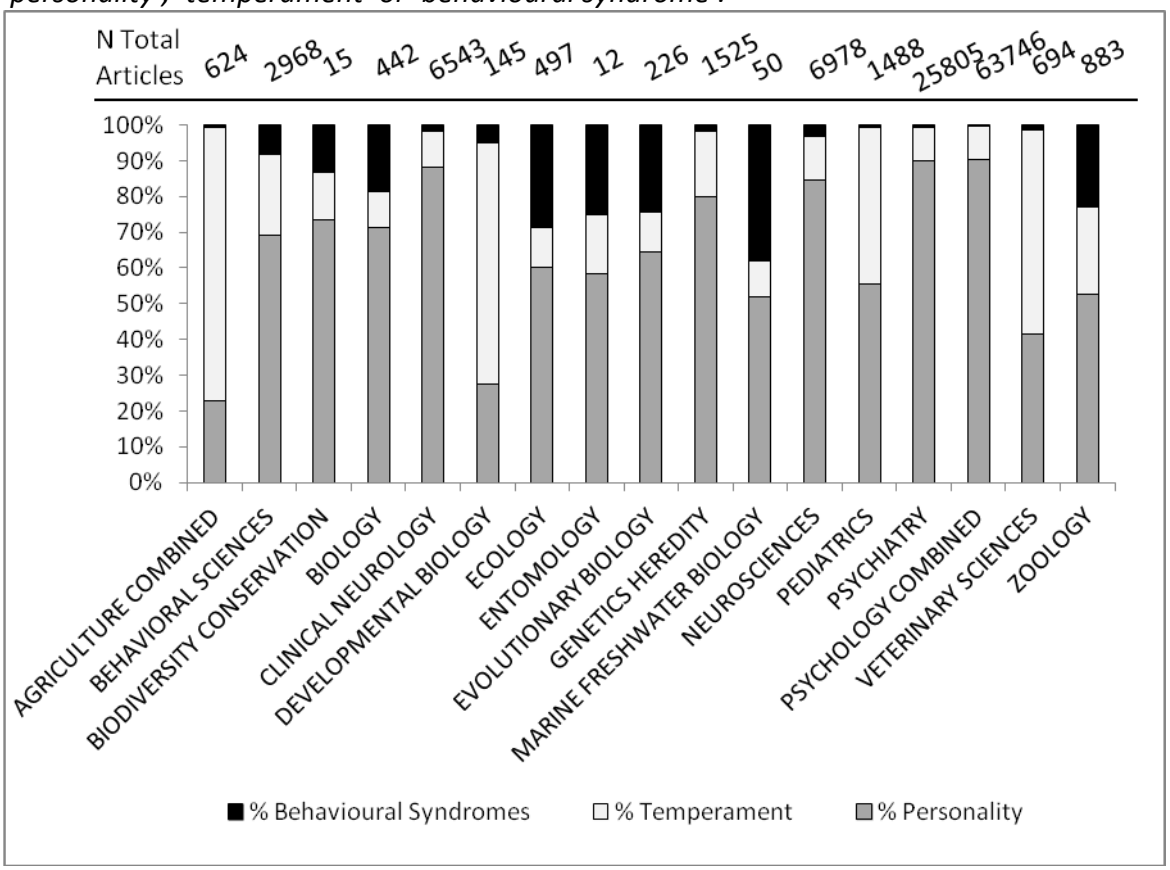
In the same review, Strelau also considered that combining the terms 'temperament' and 'personality' (in humans) made it impossible to differentiate the variance associated with only one. He argued that personality encompasses social environment, the feedback that behaviours will generate from other people, e.g. that an aggressive person will be received differently to a non aggressive person. Strelau argued that many definitions of temperament do not make this distinction. Consider the difference between discussing anxiety and fear, two similar constructs which overlap but nonetheless encompass important distinctions. Similarly when discussing child development, (Rothbart 2007) included the idea that:

> *"temperament and experience 'grow' a personality".*

If we can use the word personality in animal behaviour research, should we not also incorporate the differences between personality and temperament that already exist within the study of human behaviour? First we must address the lingering issue of whether personality is an appropriate term to use in animal literature.

Figure 2.2 demonstrated that the search term 'personality' identifies an ever increasing number of articles in ethology-related fields over the past ten years, however, we can investigate this further. Web of Science uses its own categories to group research into different fields. It is possible to investigate the distribution of articles that are identified by 'behavioural syndrome', 'personality' and 'temperament' across these categories and this is done in **Figure 2.4**.

Figure 2.4 *Percentage of articles in WoS category identified by the topic term 'personality', 'temperament' or 'behavioural syndrome'.*



Personality is the most predominant term overall but does not dominate in the fields of agriculture, developmental biology, veterinary sciences, and to a lesser extent, paediatrics. In these fields, temperament is the predominant term. Paediatrics and developmental biology are both fields concerned with early-life stages, with

developmental biology being more mechanistic. The prevalence of temperament in paediatrics and developmental sciences may be because of influential papers such as Goldsmith et al. (1987) which discuss temperament in the context of child development. The use of temperament is much lower within the fields of psychiatry and psychology compared to paediatrics, again reflecting its usage as describing child behaviour, possibly before the 'growth' of personality (Rothbart 2007; Kagan in Dugatkin 2009). Within the literature, temperament is continually described as a basic trait, an underlying trait, an early-life trait or a trait with a biological origin (McDougall 1932; Cloninger 1994; Box 1999; Watters & Powell 2011) and very much in relation to an individual and the individual's style of response to specific environmental challenges (Heffernan et al. 1982; Boissy 1995; Lloyd et al. 2008). As mentioned in Chapter One, there is also a preponderance of tests on 'fear'. This explanation does not completely account for why temperament is so prevalent in agricultural or veterinary studies. We do not expect that farm animals are more childlike than wild animals. Although there is some argument to suggest that in the process of domestication we create more juvenile animals (Goodwin et al. 1997; Price 1999) this has never been suggested as a reason why we study temperament in domesticated animals. Why then might temperament be so prevalent in these fields that do not relate to children? Firstly, 'temperament testing' has recently undergone some intensive research in agriculture and borrows many of the words from the personality literature (Wemelsfelder et al. 2001) such as bold/shy. Many behavioural assessments of welfare adapt some form of temperament test (Waiblinger et al. 2006; Forkman et al. 2007) because the consistency and repeatability of a temperament trait allow for comparison over time. This borrowing of terminology has undoubtedly

helped to confuse the issue. Additionally, 'temperament' in these instances may be inherited from the more qualitative work done discussing the behaviour of stock with the stockpeople who manage them (Dickson et al. 1970). Early usages of temperament in agricultural sciences do relate the animal's behaviour to specific contexts, for example 'milking temperament' or 'handling temperament' in cattle (Kilgour & Dalton 1984).

Regardless of why some fields use personality less than others, the fact remains (**Figure 2.4**) that personality is now a term fully incorporated into animal research, so Strelau's first argument bears less weight. Strelau & Rothbart, among other human psychologists, would argue we are losing the important variation by combining the terms. Can we address this?

2.4.5 Conclusions from literature

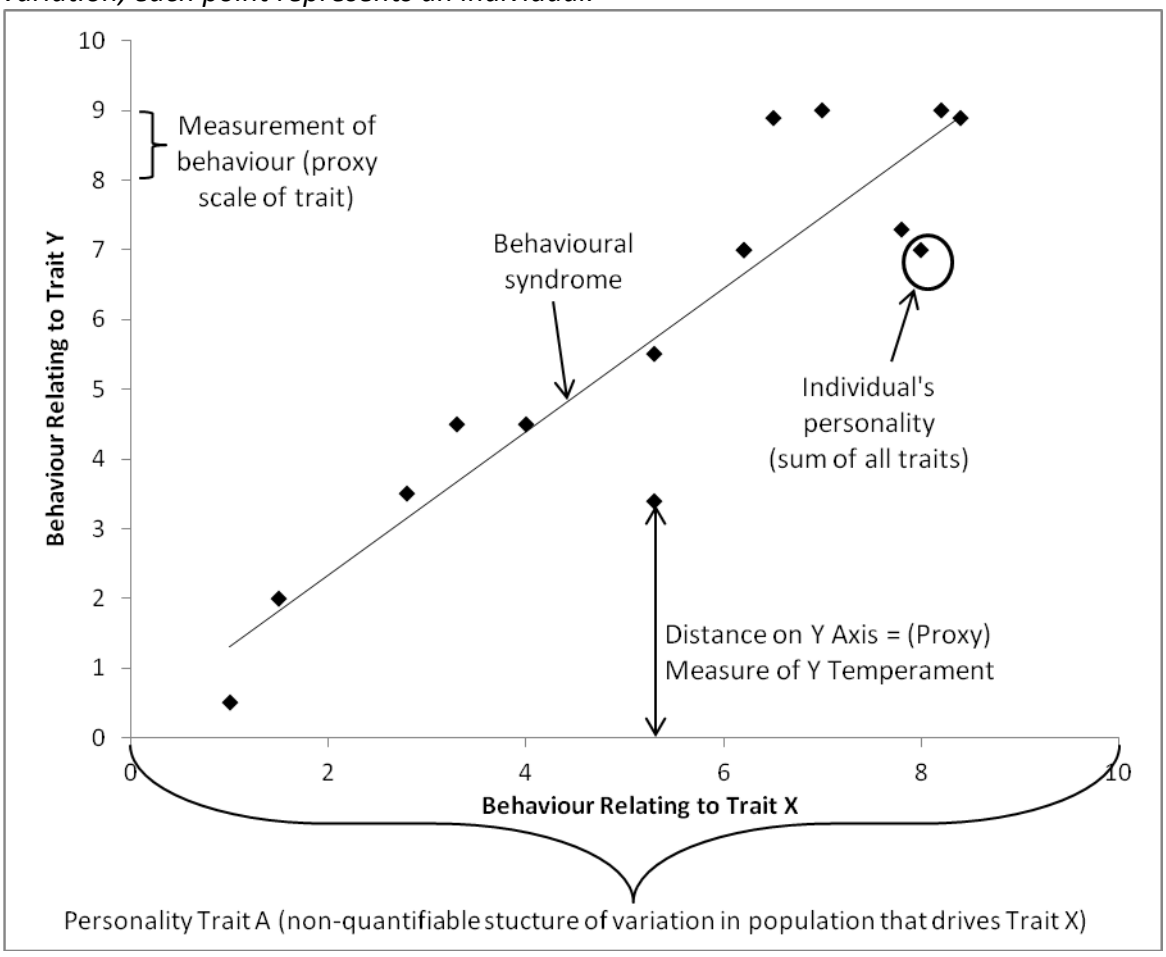
Confusion between personality and temperament almost certainly stems from the long history of wariness regarding anthropomorphism. This has helped to blur the lines between temperament and personality in ethology and methodological problems concerning the use of traits has helped to confuse the issue the further. The concept of behavioural syndromes, while addressing a new approach to the consistent individual behavioural variation, has suffered from attempting to include both personality and temperament without an appropriate framework, but clearly references a separate structure of variation from traits by discussing the relationship between traits. So how can we apply a framework to keep these terms clear?

2.5 A framework for future reference

There have been many sources of confusion between the terms, but would we be able to utilise each term uniquely in the example of the dice in **Figure 2.1**? Observant readers may note there are commonalities between **Figures 2.1** and **2.3** so let's utilise that example again in **Figure 2.5** to propose a framework for future reference when discussing behavioural syndromes, personality and temperament.

In order to choose the appropriate terminology, the researcher must ask themselves two questions. At what level is the behavioural variation being studied (i.e. between individuals, between populations or both?) and where is the consistency over time in the behavioural variation displayed (i.e. in one context or in multiple contexts?).

Figure 2.5 *Proposed framework of behavioural syndrome, personality and temperament in a hypothetical population with only two structures of behavioural variation, each point represents an individual.*



2.5.1 A working definition of personality

In an ideal world, to understand an individual's behavioural reactions, it would be tested with every possible stimulus and all its reactions would be recorded. Additionally, in this hypothetical situation, all habituation and learning events are known. The individual would then have a location in n-dimensional space, for n reactions to n stimuli, and this would be a perfect model for predicting that individual's behaviour, assuming the individual received no extra 'experience' with which to modify its behavioural responses. Similar stimuli would elicit similar

reactions. For example, a loud noise created by a siren shares many properties with a loud noise created by an explosion and we would expect a similar reaction to both from the same individual. If we then measured all other individuals in the population, we would have a model describing all the behavioural variation present in that population. Where a distribution of responses exists to the same kind of stimulus that cannot be explained by other factors such as sex, age, etc., a personality trait exists within the population, and an individual animal has a position along that trait within that distribution.

This, of course, cannot be done. Therefore, we roughly characterise the behaviour of the individual, placing the individual's behavioural response in the context of the responses of the rest of the population. Personality is the simplest model which describes the most behavioural variation in any individual, based on dimensions which encapsulate the population's behavioural variation. Therefore it is a quality of the individual, referencing the behavioural variation present in the population. It is not dependent on a particular context, which makes it all but impossible to measure directly, outside of measuring every individual in every imaginable context. In **Figure 2.5**, the personality trait is the underlying structure of variation which allows the individuals to be ranked and the individual's personality describes that individual completely.

2.5.2 A working definition of temperament

Temperament is perhaps the most difficult term to define for thanks to its long history of usage in paediatrics and agricultural sciences. Temperament is the

animal's behavioural response measured on some biological scale, such as flight speed. This definition is of more use to ethologists, who often find themselves using proxy measures and can make comparisons across populations when faced with the same context. It is this ability to quantify which makes temperament so useful, as this can be tested several times, does not require much post-hoc conversion to be understood as a trait (i.e. through factor analysis, as in personality traits) and can be related to other biological measures more easily. However, the compression of temperament test results into bivariate categories (such as good/bad) can lead to a loss of information.

In essence, this view of temperament places the individual's behavioural response in context of a certain situation and is associated with variation in the underlying personality trait within the population. For example, we talk about handling temperament, in which between-individual variation is likely motivated by the animal's underlying fearfulness when handled. This definition of temperament allows us to say that when separated from a group, the individual has a return latency of n seconds. We can use this to estimate how the animal will react to other social stimuli, but it is a measure which likely covaries along a personality dimension, not a measure of the dimension itself. In **Figure 2.5**, temperament is the ability to describe the animal's behaviour in units and is used as a proxy for the non-quantifiable aspects of personality.

2.5.3 A working definition of behavioural syndromes

Behavioural syndromes exist within populations, not individuals. They are a description of the distribution of personalities within a population. When defining personality, we discussed where clusters may form in a population, leading to personality traits present within the population. Behavioural syndromes link two traits across a population and may imply that these clusters do not allow for complete plasticity within a population. This term places the differences between individual personalities in an evolutionary context within multiple traits and populations. Behavioural syndromes reference both contexts and other populations and their behavioural variation.

In **Figure 2.5** the behavioural syndrome is the correlation between the two traits in the population. Another way of describing the individual personality could be its deviation from what the behavioural syndrome predicts.

2.6 Conclusions

This chapter has demonstrated that there is evidence for a framework for reference in the usage of the terms ‘behavioural syndrome’, ‘personality’ and ‘temperament’. By summarising the main points of confusion within the literature, the chapter clarifies where the main differences in meanings between the terms. For the rest of the thesis, the behavioural traits will be discussed in reference to the framework proposed here.

In order to investigate the possibility of assessing personality remotely, it will be necessary to understand how both temperament as a proxy measure of personality and personality traits constructed through statistical methods relate to home pen

records of activity in cattle. Therefore by utilising the definitions and framework proposed in this chapter, the thesis will remain consistent within its own usage of the various terms.

Chapter Three

Establishing the extent of behavioural reactions in dairy cattle to a leg mounted activity monitor.[2]

[2] In this chapter, I was responsible for the experimental design. I conducted the experiment, recorded and stored data, carried out the statistical analysis and wrote the manuscript.

With thanks to Emily Ullerich (a student of Iowa State University) for assistance with data collection and Ian Nevison (BIOSS) for advice on analysis.

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3.1 Abstract

Tri-axial accelerometers, typically used in activity monitors for detecting oestrus events in dairy cattle, are potentially a valuable device in the ethologist's toolbox. They open up the possibility of monitoring large numbers of animals over long periods of time with minimum human intervention. The use of such devices on domesticated cattle is widespread, however there is little systematic information available on how the animals react to being 'tagged'. Typically accelerometers are attached to a hind limb. In this study, the behaviour and feed intake of 28 lactating dairy cattle (sound n = 22, lame n = 6) was observed in housed cows for three consecutive periods: a baseline period, a period without accelerometers and a period with accelerometers. The effect of being tagged on the behaviour of the animal and whether habituation occurred within the tagged period were investigated. There was no evidence of a general change in feed intake (P = 0.438), in the proportion of time spent lying (P = 0.703) or proportion of time spent lying on the untagged side (P = 0.708) between tagged or untagged periods. All animals showed an increase in time spent standing and decrease in time spent lying over the first two tagged days, which became non significant by Day 3, when compared to the last untagged day (lying P = 0.575, standing P = 0.974), suggesting a habituation period of two days after tagging for animals to adjust to wearing the tags. From these results, the authors conclude that accelerometers are a non-invasive tool for the study of cattle behaviour, but recommend that data may not be reliable until two days after the attachment of the device.

3.2 Introduction

Recent advances in technology mean that a wide range of biotelemetry recording devices are available for many different species and purposes, such as recording activity patterns, location, or proximity to other individuals. In agriculture, a new application of activity monitors is detecting oestrus events in dairy cattle. They have been shown to be one of the most accurate methods of oestrus detection compared to the assessment of vaginal mucus resistance, mounting behaviour and other automated methods of detection such as milk yield, body and milk temperature and progesterone testing (Firk et al. 2002). Most activity monitors are now based on accelerometer technology which measures the movement of the device relative to freefall (proper acceleration). Tri-axial accelerometers, recording movement in three directions, can then provide data on the movement and activity of the subject at the point of attachment, e.g. a limb, and so function as pedometers when attached to a leg. As well as assessing movement to detect the peaks of activity that characterise oestrus, these devices can be used for other purposes. Recently, such sensors have been used to characterise the behaviour of various species in order to gain more information about individual activity patterns and movements of groups of animals (Shepard et al. 2008). If accelerometers can be used successfully to characterise the behaviour of cattle across different farms and management systems they may prove to be a useful tool when assessing welfare. For example, recording lying activity in different management systems or lactation stages (Blackie et al. 2006) can provide information on what aspects of management affect milk yield and whether stressful events, such as moving pens, can result in an alteration in activity from a baseline level. The use of such devices has become more frequent in the behaviour literature, with many validation studies run on what behaviours the devices record (McGowan

et al. 2007; Trénel et al. 2009; Nielsen et al. 2010). A recent paper showed that lying time and laterality was not affected by the presence of a pedometer (Gibbons et al. 2012). However, there are no studies that determine whether the animals take some time to adapt to the wearing of a pedometer, and whether standing and other activity is affected. As the devices are small, lightweight and less obtrusive than a human observer, there seems to be a general assumption that they have no effect on cow behaviour. For behaviour studies, if biotelemetry devices have a consistent effect on the behaviour they are recording, the results of such studies cannot be generalised to a larger population which is not fitted with the device. The present study focussed on behaviours likely to be affected by the comfort of the fit of a biotelemetry device. Activity monitors are usually attached to a hind leg in the parlour, and in the authors' experience is often met with kicking and restlessness, which may be indicators of discomfort. There is also anecdotal evidence of this continuing upon leaving the parlour after being tagged, which suggests there may be an effect of the device after the tagging procedure is completed.

The lack of guidance on when the data from an accelerometer device can be used has resulted in some variation in the literature. Previous studies have started using data from accelerometers within a few hours (Blackie et al. 2006; Aharoni et al. 2009), while others have waited for 24 hours after tagging (Bewley et al. 2010) or a fourteen hour habituation period (Gibbons et al. 2012) In some cases (Ouweltjes et al. 2009) it is difficult to discern when data was collected from the accelerometers. It appears

that most scientists assume a habituation period needs to take place, but the extent and duration of this has not been documented.

Therefore, in this study there were two objectives. The first was to establish whether a typical accelerometer (in this instance, the IceTag Pro, developed for research use by IceRobotics Ltd., South Queensferry, Scotland) has an adverse behavioural effect on a typical group of lactating dairy cattle. We also wanted to assess the effect on healthy and non-healthy animals. As lameness is a prevalent problem in UK dairy herds (Rutherford et al. 2009), a subset of lame animals was included in the study. The data was then examined within the tagged period for evidence of a habituation period.

3.3 Materials and methods

3.3.1 Animals, housing and management

Twenty-eight lactating Holstein dairy cows (lactation number 3.26 ± 1.87) were used in the study. The increasing use of activity monitors for oestrus detection means that many commercial animals will have been fitted with a biotelemetry device at some point and so the study groups included experienced (n = 22) and naïve (n = 6) animals to investigate if this affected their reaction to being tagged. Throughout the study period they were housed in a free stall barn at the SRUC Dairy Research Centre (Dumfries, Scotland) with a cow:cubicle ratio of 1:1. Each cubicle had a mattress and was bedded with sawdust. The passageways were solid concrete and cleaned by an automatic scraper every two hours. The cows were fed total mixed ration (silage, 38.95% ± 0.03%, maize 35.74% ± 0.03%, with the rest being made up

of concentrate, crimp and moist grains) *ad libitum* using automated feeders with a cow:feeder ration of 2:1. The animals were allowed water *ad libitum* and were milked three times daily at 6:30, 14:50 and 22:00. They were fed at 10:00 (with two exceptions, see Section 2.4) and were excluded from the feeders at 11:45-12:30 to allow for the automated feeder communication with the database. The activity monitor device used in this study was the IceTag Pro (IceRobotics Ltd, South Queensferry, Scotland), which has dimensions of 95mm x 85mm x 31.5mm and weighs 0.17kg. The IceTag Pro is a tri-axial accelerometer based activity monitor which samples the orientation of the device 16 times per second. This orientation data is interpreted as 'lying' and 'standing' with measures of activity such as step count arising from continuous motion recordings. The data is stored for removal later, making the devices commercial data loggers calibrated for use in cattle.

3.3.2 Experimental treatment and design

The animals were allocated into one of two treatment groups (TG1 and TG2), balanced for lactation number (TG1: 3 ± 2,2 TG2: 3.4 ± 1.5 mean and standard deviation respectively), locomotion score (1-4), days in milk (TG1 298 days ± 141, TG2 319 days ± 125, mean and standard deviation respectively) and the number of animals naïve to the IceTag device. Both treatment groups were housed together but were separated from non-test animals for the duration of the experiment. A pre-test phase prior to the test period lasted for 5 days (Days -4 to 0) to allow the group to mix. Throughout the pre-test phase, the animals were subject to dummy behavioural observations in which the observer walked through the pen with the cows and the observer acted as they would during regular observations. The subsequent

experimental phase lasted 12 days. The treatment conditions were named 'tagged', where IceTags were applied to the right hind leg, and 'untagged' where the cows were not fitted with IceTags. After an untagged baseline period of four days (Days 1 to 4) the groups were tested in the order outlined in **Table 3.1**. Treatment Group 1 were tagged for four days (Days 5 to 8), while Treatment Group 2 were untagged. On Days 9 to 12 Treatment Group 1 was untagged and Treatment Group 2 was tagged. This order controlled for effects of time. Tags were attached in the parlour at first milking on the Tagged Day 1. Observations were carried out in the same fashion from Days -4 to 12. Before the study period, all cows in the group were visually locomotion scored by a trained observer using a scale adapted from Manson and Leaver (1988) where 1 indicates a fully sound animal and 5 indicates a fully lame animal. This score was used to balance the groups. The cows were scored again by the same observer on Day -4 to assess whether the animal's condition had improved or deteriorated. Their conditions remained the same and no animals were required to be removed from the study. This score was used to assign a 'lame' or 'sound' value, with lame animals scoring 3 or 4 and sound animals scoring 1 or 2. No animals scored 5. Lameness was caused by conditions in the hoof, not of the limb itself and was present in the hind feet, not the front feet. This was discussed with experienced stockpeople and hoof-trimmers and were all considered to be typical causes of lameness, common to UK farms. Clinical causes of lameness were not recorded.

Table 3.1 *Experimental set up of treatment groups 1 and 2*

	Days -4 to 0	*1 to 4*	*5 to 8*	*9 to 12*
Treatment Group 1	Pre-Test	Baseline	Tagged	Untagged
Treatment Group 2	Pre-Test	Baseline	Untagged	Tagged

3.3.3 Automatic recording of feed data

The animals were fed using Hoko Feeders (Insentec, Marknesse, The Netherlands). These bins automatically record the weight of the feed in the bin upon the animal's approach, how long the animal feeds for and the weight of the feed on the animal's departure, thus allowing food consumption to be calculated.

3.3.4 Behavioural data

Behavioural data was recorded by instantaneous scan sampling at ten minute intervals for 90 minutes post 06:30 milking, 70 minutes post feeding, 100 minutes post feeder maintenance period and 140 minutes post 14:50 milking. The cows were therefore observed for 6 hours and 40 minutes every day in four observation periods for a total of 45 scans per animal per day. All observations were carried out by the same two observers who were familiar to the cows and who spent the pre-test period running mock observations to ensure the animals were familiar with the routine. Animals were identified by their freeze brand on the hindquarters or their corresponding management ear-tag. Together, the two observers carried out 15 training runs on all 28 animals (number of observations = 420) over three days in the pre-test period to ensure inter-observer reliability. No disagreements were recorded between the observers. The same path through the housing area was made for each observation. If an animal had not been recorded by the time the observer returned to the starting point then this was counted as a missed observation. For each observation the location of the animal, its posture and behaviour were recorded as in

Table 3.2. It was decided to record lying laterality as an additional indicator of comfort. Occasionally animals were withdrawn for routine farm procedures. In these cases the observations for those animals were scored as missing data. There was variation in the number of scans in which feeding behaviour could be demonstrated. Typically, animals could demonstrate feeding behaviour in all 45 of the scans. However, there were some occasions when the bins were locked for longer and so the animals had 3 scans less in which to demonstrate feeding behaviour, although they could still stand, lie, etc. Therefore, time spent feeding was calculated as a proportion of the time available to feed. To validate the scan sampling time, the proportion of scans lying were regressed against the percentage time recorded as lying by the IceTags. A strong correlation ($P < 0.001$, $R^2 = 0.94$) existed between the IceTag measurements and behavioural measurements, so we can be confident that the sampling time was appropriate for the postures observed.

Table 3.2 *Postural, spatial and behavioural ethogram*

Category	*Term*	*Description*
Location	Feedface	• All or some of cow's head is through the Hoko bin rails
	Passageway	• Cow is standing with at least two of her front hooves in any of the passageways within the housing area
	Cubicle	• Cow is standing with at least two of her front hooves on the cubicle mat. Alternatively, cow is lying on cubicle mat.
Posture	Standing	• At rest, no locomotion. May have one leg raised
	Walking/locomotion	• Animal moving forwards, head swings side to side, covers 1m in 5s
	Lying left	• Lying on ground, from posterior viewpoint, legs are positioned to the right of the body
	Lying right	• Lying on ground, from posterior viewpoint, legs are positioned to the left of the body
	Perching	• Standing with front feet higher than back feet, can only happen in cubicle or beside water trough.
	Other	• Misc. posture e.g. mounting
Behaviour	Feeding	• Head fully in Hoko bin, retrieving or chewing feed
	Drinking	• Head in water trough, lips submerged
	Ruminating	• Chewing or in process of regurgitating bolus
	Idle	• No obvious behavioural modifier
	Grooming self	• Cow licks self with tongue, or scratches head or other body part on surroundings
	Grooming actor	• Cow licks or rubs against another cow
	Grooming receiver	• Cow has part of body licked or rubbed against by another cow
	Other behaviour	• Miscellaneous behaviour not covered by other categories e.g. mounting

3.3.5 Statistical analysis

The aim of this study was to investigate if the change in activity from the baseline period was significantly different in the tagged period from the change from the baseline in the untagged period. The tagging order as described above controlled for any effect of a recovery period. The proportion of scans spent in each postural state, location and behaviour were calculated for each individual. The proportions for each animal were averaged by day over each treatment period. In order to find the difference from baseline observations, the means for the tagged and untagged period were subtracted from the baseline mean. The means for daily feed intake were also treated in the same manner. Using an ANOVA in GenStat (11^{th} edition, VSN International, 2010) we tested for significant effects in difference from baseline in tagged and untagged periods and an interaction between tagged status and lameness or experience. The model used was:

$$y_{ijkl} = \mu + a_i + h_j + x_k + hx_{jk} + t_l + + ht_{jl} + xt_{kl} + hxt_{jkl} + \acute{\varepsilon}_{ijkl}$$

where:

y_{ijkl} = difference of response on animal i, health status j, experience k and tagging status l from baseline for animal i.

μ = the overall mean

a_i = effect of animal i (ai i.i.d. ~ n(0, δ2A))

h_j = the effect of health status j where j=1(lame), 2 (sound).

x_k = the effect of experience, where k=1 (experienced), 2 (naïve)

t_l = the effect of tagging status (period), where l=1 (tagged), 2 (untagged).

hx_{jk} = the effect of the interaction between health status j and experience status k

ht_{jl} = the effect of the interaction between health status j and tagged status l

xt_{kl} = the effect of the interaction between experience status k and tagged status l

$\acute{\varepsilon}_{ijkl}$ = residual variation (ɛ́ijkl i.i.d. ~ n(0, δ2))

Upon inspection of the data it appeared that some states were not very frequent, such as locomotion and 'other'. Therefore only the most commonly occurring postures were analysed which were lying, standing and perching. It was assumed that behaviour may be disturbed in the period immediately after tagging. To determine the day on which behaviour stabilised, the tagged days were compared to the last untagged day (Tagged-1) in a series of sixteen paired T-Tests. This was again run in GenStat 11th edition.

3.4 Results

3.4.1 General feed intake and behavioural changes between experimental periods.

Tagging had no effect on feed intake or the proportion of scans where feeding was observed. Furthermore, there was no difference between the deviations from baseline in tagged and untagged periods in the proportion of scans observed lying, standing, perching or in the proportion of lying scans on the left hand side. The results of the analysis on all animals are reported in **Table 3.3**. Lameness and experience status had no effects and there was no interaction between lameness and experience. The results of the analysis of the different subsets are reported in **Table 3.4**. Neither naïve nor lame animals showed any difference in the proportion of scans spent feeding, lying, standing, perching, in locomotion or the proportion of lying scans on the left hand side.

Table 3.3 *Difference from baseline in tagged and untagged periods for feed intake (kg) and proportion of scans observed in each posture for all animals. Difference in means ± standard errors shown (n = 28, d.f. 1,55).*

Behaviours	*Tagged*	*Untagged*	*F Statistic*	*P Value*
Feed Intake	-1.4kg±1.92	0.6kg±1.92	0.53	0.470
Lying	0.04±0.02	0.04±0.02	0.06	0.809
Lying on left	-0.005±0.02	0.007±0.02	0.14	0.707
Standing	-0.05±0.02	-0.03±0.02	0.76	0.386
Perching	-0.03±0.01	-0.006±0.01	2.54	0.118
Feeding	-0.03±0.01	-0.017±0.01	1.01	0.319

Table 3.4 *Difference from baseline in tagged and untagged periods for feed intake (kg) and proportion of scans observed in each posture for all subgroups. Difference in means ± standard errors shown, (d.f. 1,55).*

	Tagged	*Untagged*	*F Statistic*	*P Value*
Sound (n = 22)				
Feed Intake	-2.2 ±2.17	0.3 ±2.17	0.17	0.686
Lying	0.04 ±0.02	0.04 ±0.02	0.00	0.986
Lying on Left	0.00 ±0.05	-0.01 ±0.05	1.60	0.218
Standing	-0.07 ±0.02	-0.04 ±0.02	1.86	0.185
Perching	-0.02 ±0.01	-0.002 ±0.01	1.20	0.284
Feeding	0.04 ±0.01	-0.02 ±0.01	0.75	0.395
Experienced (n = 22)				
Feed Intake	-2.4 ±2.17	0.0 ±2.17	0.08	0.779
Lying	0.03 ±0.02	0.04 ±0.02	3.71	0.066
Lying on Left	-0.03 ±0.05	-0.02 ±0.05	0.31	0.582
Standing	-0.05 ±0.02	-0.04 ±0.02	0.01	0.932
Perching	-0.03 ±0.01	-0.006 ±0.01	0.00	0.967
Feeding	-0.03 ±0.01	-0.02 ±0.01	0.23	0.635
Lame (n = 6)				
Feed Intake	1.6 ±4.19	1.5 ±4.19	0.17	0.686
Lying	0.05 ±0.04	0.05 ±0.04	0.00	0.986
Lying on Left	-0.02 ±0.09	0.07 ±0.09	1.60	0.218
Standing	0.03 ±0.04	-0.01 ±0.04	1.86	0.185
Perching	-0.08 ±0.03	-0.02 ±0.03	1.20	0.284
Feeding	0.006 ±0.02	0.007 ±0.02	0.75	0.395
Naïve (n = 6)				
Feed Intake	2.1 ±4.15	2.6 ±4.15	0.08	0.779
Lying	0.08 ±0.04	0.02 ±0.04	3.71	0.066
Lying on Left	0.07 ±0.09	0.12 ±0.09	0.31	0.582
Standing	-0.04 ±0.04	-0.02 ±0.04	0.01	0.932
Perching	-0.03 ±0.03	-0.001 ±0.03	0.00	0.967
Feeding	-0.03 ±0.02	-0.02 ±0.02	0.23	0.635

3.4.2 Variation within the tagged period.

Figure 3.1 shows the mean proportion of observations spent lying, standing and perching for all animals on the day immediately before tagging (Tagged-1) and the four tagged days. Tagged Day 1 was significantly different from Tagged-1 for lying

($T_{(27)}$ = -2.43, P = 0.022) and standing ($T_{(27)}$ = 2.49, P = 0.019) However perching behaviour did not change significantly ($T_{(27)}$ = 0.83, P = 0.415). Similar results were seen between Tagged-1 and Tagged Day 2. Tagged Day 3, however was not significantly different from Tagged-1 (lying $T_{(27)}$ = 0.57, P = 0.575, standing $T_{(27)}$ = 0.03, P = 0.974, perching $T_{(27)}$ = -0.94 P = 0.355) and neither was Tagged Day 4. Tagged Days 3 and 4 are not significantly different from each other (lying $T_{(27)}$ = 0.07, P = 0.947, standing $T_{(27)}$ = -0.08, P = 0.940, perching $T_{(27)}$ = 0.1, P = 0.923). This suggests that there is a habituation period of two days. The pattern of behaviour and significance levels for sound and experienced animals were the same. The mean proportions of observations spent in the major postures are shown in **Figure 3.2** for lame animals. Paired comparisons were made between Tagged-1 and Tagged Days 1 to Tagged Day 4. Lame animals did not show a significant difference between Tagged-1 and any of the tagged days and showed no evidence of a habituation period. **Figure 3.3** shows the same values for the naïve subset. Naïve animals showed evidence of a habituation period for standing behaviours only, with significant differences between Tagged-1 and Tagged Days 1 & 2 ($T_{(5)}$ = -2.64, P = 0.046, $T_{(5)}$ = -3.08, P = 0.027). For standing behaviour, Tagged Days 3 & 4 were not significantly different from Tagged -1 ($T_{(5)}$, = 1.23, P = 0.273, $T_{(5)}$ = -0.59, P = 0.580). Again, this suggests a habituation period of two days.

Figure 3.1 *Mean proportion of scans observed for all animals in the study for the main postures for the last untagged day and each day of the tagged period. An asterisk denotes where the value is significantly different from Tagged-1 in a paired T-test. Standard error bars are shown (n = 28)*

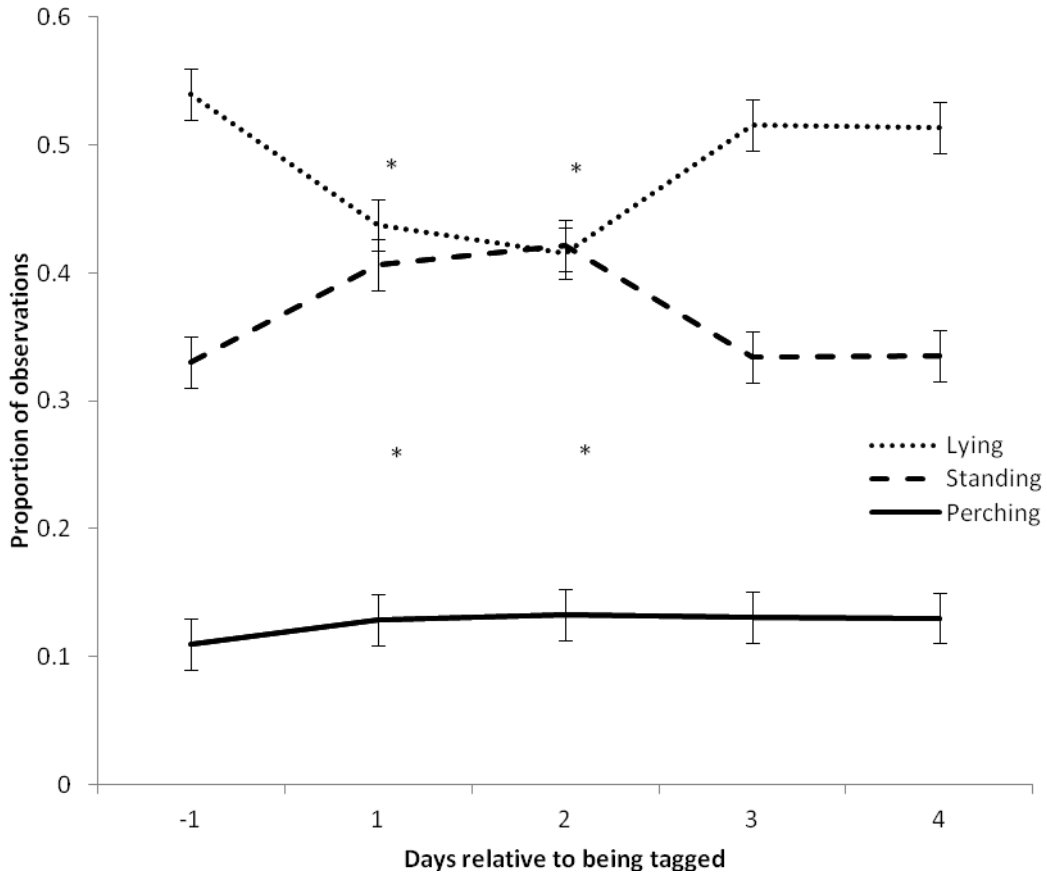


Figure 3.2 *Mean proportion of scans observed for the main postures for lame animals in the study, for the last untagged day and each day of the tagged period. An asterisk denotes where the value is significantly different from Tagged-1 in a paired T-test. Standard error bars are shown (n = 6)*

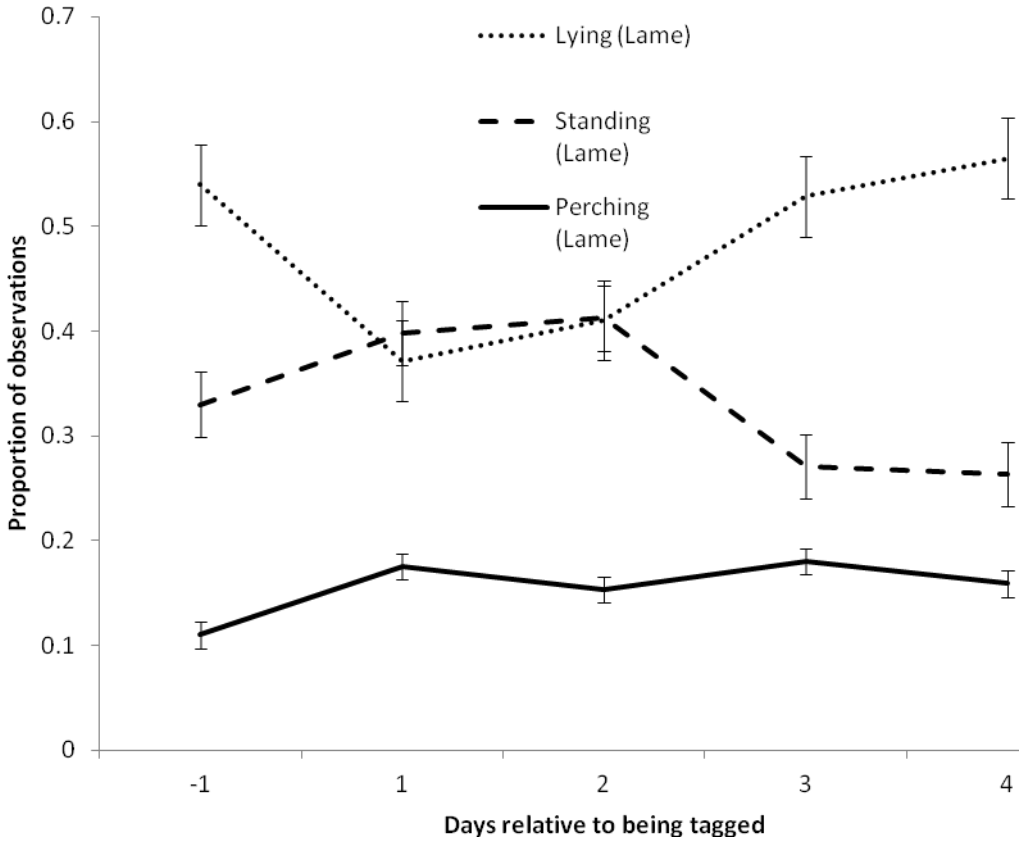
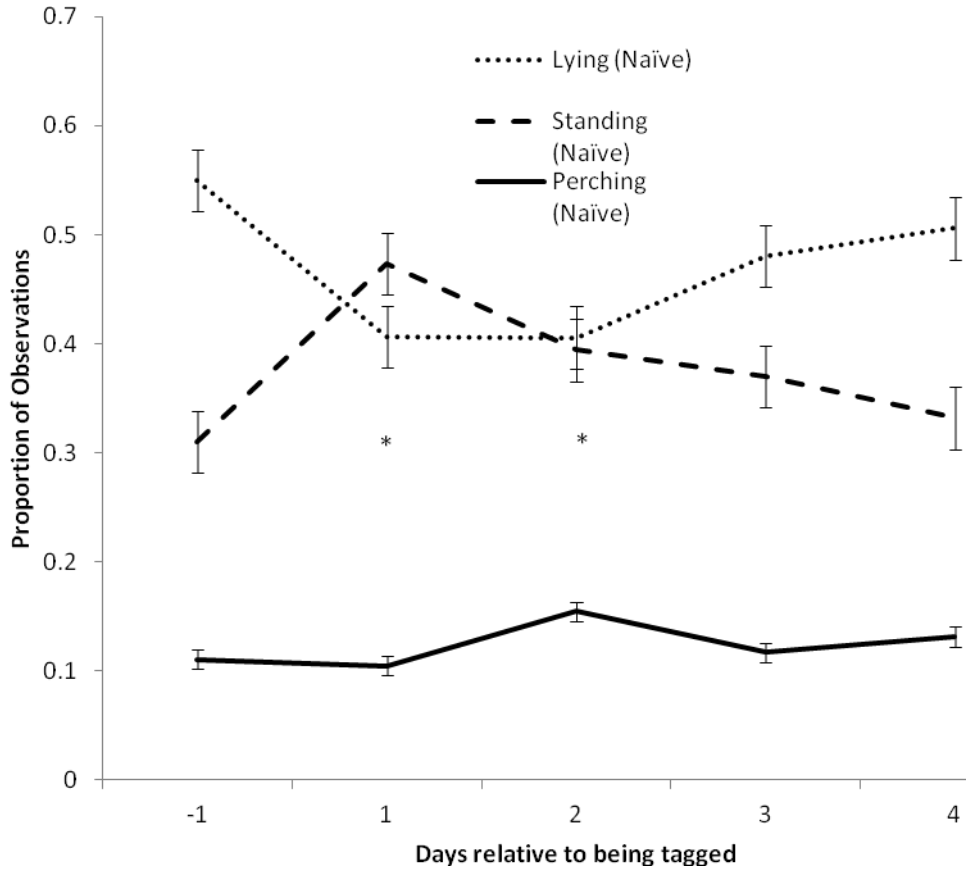


Figure 3.3 *Mean proportion of scans observed for the main postures for the naïve animals in the study, for the last untagged day and each day of the tagged period. An asterisk denotes where the value for standing is significantly different from Tagged-1 in a paired T-test. Standard error bars are shown (n = 6)*



3.5 Discussion

Overall, it appears that IceTags, or other similar devices, are a suitable, non-disruptive method of recording behaviour and have no obvious effect on the behaviour of cows after a short period of habituation. No significant general change was seen in the time spent standing, lying and feeding when the device was fitted.

This study found no evidence of animals changing the amount of time spent lying on their left hand side when tagged. Laterality preferences vary with age and pregnancy state (Arave & Walters 1980) and so a change in laterality after being tagged may have suggested discomfort. This supports the conclusions found by Gibbons et al., (2012). Naïve animals were as capable of adapting as experienced animals. Although no significant effect was found in lame animals, there was also no evidence of a habituation period, which may suggest that with more lame animals an effect may have been found. If further studies are concerned with activity monitors affecting the behaviours of lame animals, they should determine the affected limb, clinical causes of lameness and vary the point of attachment. This effect is likely to be caused by discomfort as lameness in cattle can result in significantly lower nociceptor thresholds than non-lame animals (Whay et al. 1998) and not due to other reported effects of biotelemetry tagging, see below. From examination of the observed behaviours during the tagged period, the authors recommend a habituation period of two days from the tagging event. This will ensure that the animals have time to adjust to the device. The lack of evidence for a habituation period in lying behaviour for naïve animals is probably due to the larger proportion of time that animals spend lying than standing and the small sample size of naïve animals, which makes the difference harder to detect. However, the habituation period of two days remains true for standing behaviours. Problems with comparing studies using different methodologies and IceTags have already been highlighted by (Tolkamp et al. 2010) who established a criterion for defining lying bouts recorded by IceTags. A standardised methodology as suggested here for the use of biotelemetry devices in

agriculture will be invaluable for the expansion of these new tools, allowing for comparison across studies.

Biotelemetry is often used in conservation and ecology work to gather information about behaviours or population distributions. The use of these devices on wild animals has highlighted the most commonly encountered problems, such as capture stress, the effects of the experiment procedure, attachment methods and effects of equipment on fitness (Casper 2009). The effects of biotelemetry devices on fitness can be categorised into three main areas: the weight of the device, the appearance of the device, and the comfort of the device (Hawkins 2004). In other words, does the device incur a fitness cost by asking the animal to carry it, does it present an alarming visual, olfactory or other sensory cue to the animal, companions or predators, or does the attachment of the device restrict or in some other way influence the behaviour of the animal? For instance, an attachment on the leg may influence the animal's desire to lie down. Discomfort caused by a device may affect any number of behaviours (Ropert-Coudert & Wilson 2004). Mice implanted with intra-abdominal biotelemetry devices showed significant changes in climbing, locomotion and eating behaviours after implantation, greater even than in sham operated animals (Baumans et al. 2001). The authors suggested that this was due to the greater discomfort of the implant versus the discomfort of the operational procedure alone. Comfort is not the only possible factor to influence behaviour of tagged animals. The weight of an activity monitor, or other biotelemetry device, carries an energetic cost which may influence the choices made by the animals. When considering possible effects of biotelemetry on cattle, not all of these problems

apply. Capture stress is not an issue as dairy cattle are handled several times a day for milking. Cattle are also accustomed to seeing brightly coloured tags on the ears of other animals which appear to present no alarming visual cue. (Swain 2003) tested the effect of the colour of a stationary aerial on the grazing behaviour and found that the animals were not affected by the different colours. This suggests that the bright colouration of any activity monitor is analogous to other items on farm and unlikely to be alarming. Although we do not know what olfactory cues such devices present, the materials are non-toxic, ruggedized for a farm environment and are not dissimilar to other types of tag, such as a milking parlour transponder. While the weight and appearance of activity monitors are unlikely to have an effect on the animals, the concern addressed with this study is that the fitting process or the lack of symmetry caused by being tagged on one side does not have an effect on behaviour.

3.6 Conclusions

From this result, we can be confident that activity monitors mounted on the legs of cows do not affect overall behaviours and so they are a valuable tool for studying the behaviour of cattle. Results gained from tagged populations can be extended to untagged populations. By establishing the habituation period of two days, these methods can be standardised across different studies.

Chapter Four

Short term temperament tests in beef cattle relate to long term measures of behaviour recorded in the home pen[3]

[3] In this chapter I was partially responsible for experimental design. The set up of pens and diet and balancing of the animals within the pen was designed by Jimmy Hyslop. I was responsible for the addition of temperament testing to the experiment and the design of this addition. I also collected the temperament test data, activity data, collated the feeding data, conducted the statistical analysis and wrote the manuscript.

With thanks to Mhairi Jack, Simon Turner and Marie Haskell for help collecting data on farm during the temperament tests and particularly Mhairi for downloading the IceTag data. Ian Nevision and Mintu Nath from BIOSS advised the statistical analyses.

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4.1 Abstract

Handling temperament tests for beef cattle have been related to production traits, with calmer temperaments showing greater growth rates. In most tests of temperament or personality, the observation of the animal takes place over a short period of time, sometimes completed in a matter of minutes. This study investigated whether the behaviour observed in a temperament test was reflective of the steer's behaviour in the home pen. Sixty seven indoor-housed, cross-bred *Bos taurus* beef steers were fitted with tri-axial activity monitors (IceTags, IceRobotics Ltd.) and activity was recorded for two periods of 14 days each. Each steer was also scored on four measures of temperament; two handling tests (flight speed and crush score) and two feeding behaviour scores (aggression at the feeders and ability to displace at the feeders). Each temperament observation was repeated four times with repeatability of the traits ranging from 0.23 for aggression to 0.48 for flight speed. Activity measures derived from the accelerometer data, such as bout lengths, were found to be highly repeatable between the two periods of activity monitoring (repeatability of average lying bout duration 0.67, and of average standing bout duration 0.70). Steers with a high flight speed also showed higher levels of activity in the home pen (MotionIndex $r_s = 0.35$, $P = 0.004$, average step count $r_s = 0.34$, $P = 0.005$) than steers with a low flight speed. Steers which were more capable of displacing other steers at the feeders had a longer average standing bout duration ($r_s = 0.26$, $P = 0.036$), were more variable in their total standing duration (standard deviation of standing time $r_s = 0.27$, $P = 0.030$) and lay down for less time overall ($r_s = -0.35$, $P = 0.004$). No correlations were found between aggression at the feeders or crush score and home pen behaviour. This is the first time short-term tests of temperament have been found to

relate to longer term behaviour data in beef steers, as assessed by activity monitors. These results should be taken into consideration when selecting temperament tests for future use.

4.2 Introduction

Stock persons, veterinarians and those who work with cattle often speak of individual animals as being 'fearful', 'good-tempered', 'aggressive' and many other descriptions of how cattle respond to challenging situations such as interactions with people or other animals. Scientists often quantify these characteristics through 'temperament assessments', short term tests of behaviour in response to a standardized stimulus. It is generally assumed that these tests inform in some way on the underlying components driving behavioural variation however it is not known how these traits relate to how cattle behave when away from humans. In spite of this, calm or docile temperaments, as defined by these tests, can predict production traits. Average daily weight gain has a negative relationship with flight speed from a crush (Burrow, 1997, Müller & von Keyserlingk 2006) and the steer's reactivity to being held in a crush (CS) (Voisinet et al. 1997b). If these short term personality tests are only measuring something relevant to the immediate period in which they are conducted, then we must assume that handling periods have a huge effect on cattle farming and meat production. It is more likely that these personality tests, measured in the short term, reflect an underlying trait which continues to influence the animal's behaviour in other ways, going on to affect production traits, such as average daily gain. The previously mentioned tests are used in beef cattle to describe their reactions to the handling experience of being in a crush. Beef cattle have a general

reactivity to handling which is consistent and has a genetic component (Grignard et al. 2001), and in this context-specific situation should be referred to as a temperament trait (Réale et al. 2007). Aggression and dominance are also thought of as traits which influence cattle behaviour. As well as the animal's handling reactivity, the relationship between conspecific directed aggression (such as aggression at a feedface), the animal's ability to displace other animals (dominance) and other behaviours is not clear. *Bos taurus* beef castrated males are usually finished for slaughter at around 18 months and will likely spend two winters kept inside and fed concentrate in order to improve their weight gain (Phillips 2001). Being castrated, they are considerably less aggressive than intact or vasectomised bulls when kept in mixed groups (Albright & Arave 1997) as hormones play a large role in modulating aggression. Although the truly wild ancestor of cattle is not available for study, research on feral cattle groups has shown that males tend to associate in loose clusters with other adult males (Lazo 1994; Bouissou et al. 2001). As steers will spend a considerable amount of time in a home-pen in all-male groups, it is important to know more about what affects their behaviour in this context, not just in handling and feeding. In this respect, these temperament tests are simply one way we can attempt to quantify the underlying dimension which dictates how the individual will respond to challenging situations. In order to understand this, we need to more fully examine what these personality tests are measuring and how that relates to the animal's life.

With recent developments in remote sensors it is possible to remotely characterize certain elements of cattle behaviour unobtrusively and with great accuracy and

specificity (Nielsen et al. 2010; MacKay et al. 2012) allowing us to monitor day-to-day activity when cattle are not under direct human observation. With this technology we can begin to estimate how the traits measured by temperament tests reflect how cattle choose to budget their activity. To do this, we need an understanding of what activity traits are informative and how they are affected by temperament. Four temperament tests were chosen, adapted for use in cattle (Burrow 1997; Gibbons et al. 2009b) measuring feeding aggression, feeding dominance, handling temperament and motivation to leave handling area. The aggression and displacement indexes correlate with aggressive behaviours displayed and so show convergent validity, but to my knowledge they haven't been found to have discriminant validity. Turner et al. (2011b) found that flight speed and crush score correlated within a group of steers, showing convergent validity as both are considered to reflect fear of handling. Furthermore, flight speed tests do not correlate with an isolation score (although crush score does) which may suggest discriminant validity for flight speed. The validity and repeatability of these tests made them suitable for inclusion in this project. It was hypothesized that if the temperament tests measured an underlying general trait correlations would exist between the short tests and the home pen activity. If the temperament tests measured a context-specific trait, there would be no relationship with home pen behaviour. This could have implications the use of these tests as a proxy for general behaviour, influencing their use in welfare assessment and as selection criteria.

4.3 Materials and methods

4.3.1 Animals, housing and test area

The study was carried out at the SRUC Easter Howgate beef unit for 57 days in autumn 2010. The test animals were 72 beef steers with an average starting weight of 495.7kg ± 39.12 and age of 474 days ± 16 (mean ± S.D.). Five steers were excluded from the dataset due to missing data, lameness or failing to adapt to the feeders and thus leaving the trial early, resulting in an analysis based on 67 steers. The steers were reciprocal crosses. Steers with a purebred Aberdeen Angus sire and an Aberdeen Angus cross Limousin dam were designated AAX (n = 38), while steers with a purebred Limousin sire and a Limousin cross Aberdeen Angus dam were designated LMX (n = 29). All steers were from the same nine sires with offspring balanced between the four pens (AAX n = 5 sires, LMX n = 4 sires). The steers were placed in four groups balanced for weight and breed. Each group was assigned to an identical pen of approximately 18m × 9m, bedded with straw. There were eight automatic bin feeders (Hoko bin feeders, sometimes marketed as Insentecs, Insentec B. V., the Netherlands) and a water trough per pen. Two bins per pen contained straw and six contained concentrate feed. There was a ratio of 1:3 feed bins and 1:9 straw bins to steers. In each pen two CCTV video cameras were installed above the feeders to monitor feeding behaviour. Each pen was fed a barley based diet as part of a separate experiment, based on a 2 × 2 × 2 factorial design, one factor being breed, either with or without partial replacement of barley with glycerol and either with or without a yeast based probiotic (Actisaf). The diets were balanced for palatability and protein content and the diet (hereafter referred to as ‘pen’) was accounted for in the analysis (see below). The diet was not expected to have any effect on home pen

behaviours or temperament. The handling set-up featured a semicircular race which fed into a squeeze crush.

4.3.2 Crush Score and Flight Speed

When the steers were being temperament tested, the squeeze crush exited into a 6m long race. After moving through the race, the steers were moved into a post-test holding pen and then returned to their home pen once testing was complete. Two laser sensors were connected to a timer. The first laser sensor was set 1m from the crush opening which allowed for the crush bail to open, for the steer to toss its head when confined without setting off the sensor before the start time was recorded. The second laser sensor was 4m further along the race which stopped the timer. Two assessments of handling temperament were made: a crush score and flight speed test. This happened on alternate Mondays during the test period at the same time as the steer's weekly weighing. Steers were moved as a pen group from their home pen to a holding pen where they were then encouraged by experimenters or stockpeople through a semi-circular race to a squeeze crush, which also contained a weigh scale. The steers were tested on day 15, day 29, day 43 and day 54 of the trial. The test procedure was as follows and the pen weigh-order was alternated each week. Each steer was held in a crush and given a crush score by the same observer for all four test days. The crush score procedure and ethogram were adapted from Turner et al. (2011b). The steer was confined in the crush and its head secured in the bail. The squeeze mechanism was not applied. The handlers stepped away and the observer monitored the steer for signs of restlessness for a count of ten seconds to arrive at a score based on the ethogram shown in **Table 4.1**. A weight was recorded and the

steer was subject to leg manipulation to either remove or apply an activity monitor (see below). The steers were spray-painted using tail paint to mark them for the videos and released directly into the straight race. When the steer was released, the laser sensors started and stopped the electronic timer, resulting in a flight time from which a ms^{-1} score for its flight speed from the crush was calculated.

Table 4.1 *Ethogram for categorising behavioural reaction to being held in squeeze crush.*

Behaviour	Resulting Crush Score
Animal remains steady in crush, no shifting of weight and no movement of legs	1
Occasional and gentle shifting of weight.	2
Straining at the bail is seen	3
Straining at the bail is seen, plus head throwing. Crush may shake.	4
Violent and continual shaking of the crush. Animal lunges back and forth and may fall during escape attempts.	5
Animal is dangerous or unmanageable. Reading ear tag may risk handler injury. Animal may fall during escape attempts. Crush would move if not anchored.	6

4.3.3 Aggression and displacement at the feeders

An ethogram from Gibbons et al. (2009) was adapted for use at the Hoko feeders based on initial video observations (**Table 4.2).** Hoko feeders are bins approximately 1.20m wide each. The bins are designed so that only one steer may feed from a bin at any one time. Access to the bins is allowed through a 'v'-shaped head and neck space, much like a typical yoke feeder in a dairy herd set-up. When unoccupied, this access point is blocked by a raised 'door' which comes up to the top of the 'v', prohibiting access to the bin. Each steer has an infrared tag on his ear which identifies them individually and thus allows the bins to record which steer is eating and how much is being consumed. When the steer wants to feed, he pushes his head through the 'v' and this cuts a beam at the bin's entrance, dropping the 'door' to allow the steer's head access to the bin. The steer's body remains in the home pen. To be recorded as occupying a bin, the steer's head and neck was required to be through the bin door with the bin door down. As this set up is designed to ensure that

only one steer could occupy a bin at any one time, aggressive interactions often occurred behind the feeding steer. To account for this, interactions were considered to be 'at the feeders' when the front feet of both steers involved were within one steer's length of the bins. Initial analysis of the Hoko data showed that the period for two hours after feeding was the busiest and so this was chosen as the sample period for the aggression and displacement at the feed face studies. The bins were typically filled at 0800 hours. Daily intake (kg) and duration feeding (seconds) were calculated for each steer based on Hoko records. Erroneous data, such as records featuring negative intakes or feeding bouts lasting less than 10s or longer than an hour were removed. These daily totals were averaged across the study period. An aggression index was adapted from Barroso et al. (2000) by determining how often the steer acted aggressively or was the recipient of aggression. This resulted in a final score from 0 to 1, with 0 being a steer who was always receiving aggression at the feeders and 1 being a steer which was always giving aggression at the feeders.

$$Aggression\ Index = \frac{no.of\ times\ aggressor}{no.of\ times\ aggressor + no.of\ times\ receive\ aggression}$$

A displacement index based on one used by Galindo & Broom (2000) was calculated by recording the number of displacements (the complete withdrawal of the recipient steer's head from the Hoko bin following an aggressive interaction from another steer (aggressor), resulting in disrupted feeding from the recipient). This resulted in a final score from 0 to 1 where 0 represents a steer who was always being displaced from the feeders and 1 being a steer who always displaces others at the feeders.

$$Displacement\ Index = \frac{no.of\ active\ displacements}{no.of\ active\ displacements + no.being\ displaced}$$

Observations were taken from the CCTV footage for two consecutive days at four intervals, with a fortnight between each interval. These were at experimental day 10 to day 11, day 23 to day 24, day 37 to day 38 and day 50 to day 51. The observations were all carried out by one observer. An intra-observer reliability test on four hours representative of the whole sample period showed good concordance throughout as judged by Lin's concordance of correlation coefficient (0.93) and so no further data on intra-observer reliability will be presented. In addition, the various aggressive categories detailed in the ethogram were calculated as a proportion of behaviours shown at the feeders and compared with the steer's aggression index and displacement index.

Table 4.2 *Ethogram of behavioural categories used to calculate the aggression index and displacement index.*

	Category	Description
Aggressor		
Pushing	Contact	The steer uses some part of the body *other* than the head to displace recipient
Butting	Contact	The steer uses head to head, head to neck, or head to flank contact to displace recipient
Bulldoze	Contact	The steer forcefully enters the Hoko bin to displace individual
Threatening	Non Contact	The steer takes up a threatening posture by presenting the head in the direction of recipient, but no contact occurs
Recipient		
No response	Non Responsive	Steer shows no physical response
Avoids	Active Avoidance	The steer does not occupy a Hoko bin and moves in opposite direction to avoid aggressor
Withdraws back	Displaced from feeder	The steer withdraws head from Hoko bin and moves away from interaction
Retaliates (loss)	Aggressive Responsive	Steer retaliates with an attack (e.g. butt, push, etc.) towards aggressor, but ultimately withdraws from the Hoko bin.
Retaliates (win)	Aggressive Responsive	Steer retaliates with an attack towards aggressor and further aggressive interactions follow. The aggressor does not succeed in entering the Hoko bin.

4.3.4 Activity monitoring

Each steer's activity was recorded with an IceTag Pro (IceRobotics Ltd, South Queensferry, Edinburgh, UK) for two 14 day periods within the 57 day study, with a 14 day break between removal of the tags on the first occasion and re-application on the second. IceTags are tri-axial accelerometers which function predominantly as pedometers when attached to the leg of a cow. Each device has dimensions of 95mm x 85mm x 31.5mm and weights 0.17kg. The tag samples the orientation of the device 16 times per second (which is then interpreted as 'lying' or 'standing') and calculates

activity as MotionIndex; a measure of absolute acceleration over a given period, from which a step-count is derived (see **Table 4.3**). MotionIndex, and thus the step-count, recorded by IceTags are proprietary algorithms of IceRobotics Ltd. but have been found to be both accurate and specific (Nielsen et al. 2010). Tags do not affect cattle behaviour (MacKay et al. 2012). Thirty IceTag Pros were available for use on the 67 test steers. To ensure all steers were monitored evenly within each pen, steers were assigned to one of two groups, A or B. This was for tagging purposes only and did not affect the steer's management and the A and B groups were balanced for live weight and breed. In all pens, the A groups were tagged from day 1 to day 14 (Period A1) and day 29 to day 42 (Period A2). The B groups were tagged on day 15 to day 28 (Period B1) and day 43 to day 53 (Period B2). Any days with partial data, e.g. tag removal or application days, and the first two tagged days of each period were removed from the dataset, as suggested in MacKay et al (2012). Data were downloaded from the IceTags with IceTagAnalyser. This study was also interested in what home pen activity measures might relate to temperament traits so both measures taken directly from the tags and derived measures were examined. Direct measures were MotionIndex, time spent lying, or step count. Derived measures were the number and distribution of lying bouts, using an adapted version of the program used by Tolkamp et al. (2010) which used the same criteria of a minimum lying bout duration of four minutes to discern true lying bouts from artifactual lying bouts. Further derived measures were activity measures per bout or per minute standing. The standard deviations of some traits were also calculated as a measure of variability. A full list of activity traits and their method of calculation can be found in **Table 4.3.**

Table 4.3 *Measures derived from activity recorded by IceTags in 67 cross bred beef steers. Every variable is expressed as an average over the number of days on which activity data was recorded. Times are expressed in minutes; step-count and MotionIndex are continuous variables with no units.*

Basic Measure	Description
Daily MotionIndex	Proprietary measure of IceRobotics: a measure of acceleration over a given period. Can be thought of as a proxy measure of energy of movement in all three planes.
Daily Step Count	Step count is calculated by IceTagAnalyser, based on MotionIndex. This is only calculated when the tag is recorded as being in a standing position.
Total Daily Lying Time	Time the tag spent in a lying position, taken from IceTagAnalyser[1]
Lying Bouts	The following traits were calculated based on the criteria established by Tolkamp et al. (2010).[2]
Average lying bout duration	The average duration of all lying bouts recorded on a daily basis.
Average minimum lying bout duration	The average duration of the shortest lying bout recorded per day.
Average maximum lying bout duration	The average duration of the longest lying bout recorded per day.
Lying bout number	Number of true lying bouts started within a day[3]
Standing	Time the tag spent in a standing position, taken from IceTagAnalyser[1]
Standing Bouts	The following traits were calculated based on the criteria established by Tolkamp et al. (2010).[2]
Average standing bout duration	The average duration of all standing bouts recorded on a daily basis.
Average minimum standing bout duration	The average duration of the shortest standing bout recorded per day.
Average maximum standing bout duration	The average duration of the longest lying bout recorded per day.
Standing bout number	Number of true standing bouts started within a day[3]

MotionIndex per Standing Bout	A putative measure of activity in each standing bout.
Steps per standing bout	A measure of the animal's activity when standing only.
MotionIndex per minute standing	Total MotionIndex by total minutes spent standing as an indicator of general activity compared to time spent in a standing position.
Steps per minute standing	Total step-count by total minutes spent standing as an indicator of activity while standing.
Standard Deviations	
S.D. of Step Count	The individual's standard deviation of their daily step count as an indicator of the steer's variability in activity
S.D. of Daily Lying Time	The individual's standard deviation of their daily lying time as an indicator of the steer's variability in activity
S.D. of Daily Standing Time	The individual's standard deviation of their daily standing time as an indicator of the steer's variability in activity

[1] Total standing time and total lying time are strongly related as the tag accounts for all time as either standing or lying.
[2] Lying bout duration and standing bout duration are not computationally related.
[3] The final bout did not have to finish within that day, i.e. a bout which lasted over midnight was assigned to the day it began in.

4.3.5 Statistical analyses

4.3.5.1 Repeatability of behavioural traits

The four temperament traits were each measured at four intervals. The repeatability of each personality trait was calculated using the variance method as described in Gibbons et al. (2009) with linear mixed models using REestricted Maximum Likelihood (REML) and fitting steer ID and repeat as the random effects, and using the variance components in the following calculation as in Lessells & Boag (1987).

$$Repeatability = \frac{Variation\ between\ animals}{Variation\ between\ animals + Variation\ within\ animal}$$

This method was also used to calculate the repeatability of the activity traits between the two periods in which activity was recorded. As repeatability estimates have not been found to relate to number of repeats (Bell et al. 2009a), this was considered acceptable. Spearman rank correlations were then used to examine the relationship between different activity traits to evaluate which were most useful to include in the next stage of analysis.

4.3.5.2 Characterizing the relationship between activity and temperament

Here we investigate whether these four short term temperament tests reflect underlying variables which also drive variation in home pen behaviour over a longer period of time. Median and first and third quartiles are shown for each temperament score and main activity traits are shown in **Table 4.4**. Flight speed and crush score were judged to be non-normally distributed by a Shapiro-Wilk test (flight speed W = 0.95, $P = 0.008$, crush score W = 0.96, $P = 0.028$). Aggression index and dominance index were normally distributed ($P > 0.05$). Preliminary analyses used linear mixed models. We ran separate models for each temperament trait as there were some relationships between them. The models used each of the four temperament scores as a fixed effect to explain the variation in the activity traits listed in **Table 4.3**. Breed was included as a fixed effect and pen was included as a random effect in each model. These analyses did not find that breed or pen improved the models. The power of linear mixed models is that they can accommodate multiple effects on an outcome variable. As neither of the obvious parameters had any statistically significant influence, it was decided that Spearman rank correlations were the simplest way to characterize the relationship between short-term temperament tests

and long term home pen activity. As a nonparametric correlation, this has two advantages, first by dealing with the non-normal distributions of some traits and secondly by not assuming that a given trait drives variation in the other. All analyses were carried out in GenStat (version 14).

Table 4.4 *Repeatability estimates, estimated variance components between animals and within animals for temperament test and activity bout data recorded in 67 cross bred beef steers.*

	Temperament Traits						
	Flight Speed (ms^{-1})	Crush Score	Aggression Index	Displacement Index			
Median	1.19	2.25	0.49	0.50			
Q1	1.01	1.75	0.42	0.39			
Q3	1.57	3.00	0.55	0.57			
Standard Deviation	0.47	0.88	0.10	0.15			
Estimated variance component							
Between animals	0.17	0.53	0.006	0.02			
Within-animal	0.19	0.95	0.02	0.03			
Repeatability (between four tests)	0.48	0.36	0.23	0.37			
	Activity Traits						
	Mean Daily N Lie Bouts	Mean Lie Bout (mins)	Mean Min Lying Bout (mins)	Mean Max Lying Bout (mins)	Mean Stand Bout (mins)	Mean Min Stand Bout (mins)	Mean Max Stand Bout (mins)
Median	12.45	71.55	5.55	192.00	45.61	1.55	200.00
Q1	11.00	60.64	4.48	166.90	37.84	0.58	16.40
Q3	14.55	78.65	7.61	222.50	53.34	2.75	236.20
Standard Deviation	3.45	12.80	2.61	36.52	12.24	1.43	49.16
Estimated variance components							
Between animals	8.83	139.06	1.05	951	127.49	1.68	1706
Within-animal	10.04	69.97	13.59	1190	54.83	1.10	2139
Repeatability (between two tagged intervals)	0.47	0.67	0.07	0.44	0.70	0.60	0.44

4.4 Results

4.4.1 Repeatability of trait responses and activity bouts

The overall repeatability of the temperament traits between sample periods was good (**Table 4.4**). Aggression index had the lowest repeatability at 0.23, while flight speed had the highest repeatability at 0.48. Activity bouts were also repeatable between two tagged intervals, with the measures derived from the bout calculation method proving to be moderately to highly repeatable from between 0.44 to 0.70. The exception to this was average minimum lying bout, which had a repeatability of 0.07.

4.4.2 Relationships within temperament and activity traits.

Aggression index and displacement index were highly correlated (r_s = 0.79, P < 0.001) so steers with a high aggression index were also likely to be able to displace other steers effectively. The aggregated aggression index score did not explain much of the variation in the steer's proportion of aggressive responses to attempted displacements in a linear regression (R^2 = 0.05, P = 0.037) but steers with a high aggression index were significantly more likely to attempt to displace another steer (R^2 = 0.31, P < 0.001). Crush score and flight speed were also significantly correlated (r_s = 0.34, P = 0.005). Neither aggression index nor displacement index bore any relationship with flight speed or crush score (P > 0.05). This suggests that the traits measured by CS and FS and feeding behaviour, are not directly related.

Average lying bout duration was positively correlated with both minimum (r_s = 0.42, P < 0.001) and maximum lying bout duration (r_s = 0.72, P < 0.001), which suggests that steers with a long average lying bout duration tended to have longer lying bouts overall, rather than a few exceptionally long lying bouts driving up the average. This

is supported by the negative correlation between average lying bout length and the number of lying bouts in a day (r_s = -0.91, P < 0.001). Therefore the mean bout durations are likely to be as informative as the maximum and minimum bout durations. Likewise, a high average standing bout duration was associated with high minimum (r_s = 0.45, P < 0.001) and maximum standing bout durations (r_s = 0.56, P < 0.001). Average lying bout and average standing bout length were also correlated (r_s = 0.63, P < 0.001); hence steers with longer average bout lengths tended to have longer, fewer bouts in general.

4.4.3 Relationships between temperament and activity

4.4.3.1 Flight speed and crush score

A correlation matrix of home pen activity, feeding behaviour and temperament traits is shown in **Table 4.5**. Flight speed correlated positively with the average daily MotionIndex (r_s = 0.35, P = 0.004) and average daily steps (r_s = 0.34, P = 0.005). Unsurprisingly, steers with a fast flight speed scores also had higher MotionIndex and step counts per minute standing (MotionIndex/minute standing r_s = 0.29, P = 0.020, steps/minute standing r_s = 0.27, P = 0.025) but their standing bouts in general were not more active (MotionIndex/standing bout r_s = 0.16, P = 0.234, Steps/Standing bout r_s = 0.23, P = 0.066). Steers with fast flight speeds also tended to be more variable in their step count, with flight speed being positively correlated with the standard deviation of step count (r_s = 0.24, P = 0.055). There were no relationships between crush score and home pen activity (P > 0.05). Flight speed also had a negative correlation with the average daily kg consumed from the Hoko bins (r_s = -0.24, P = 0.047), although crush score showed no relationship (P > 0.05). Fast

steers therefore ate less than their slower companions. Neither flight speed nor crush score had any relationship with the daily average time spent feeding ($P > 0.05$)

Table 4.5 *Spearman rank correlations between home pen behaviours and four temperament traits in 67 cross bred beef steers. Activity traits measured by IceTags, times are expressed in minutes; step-count and MotionIndex are continuous variables with no units. Feeding behaviour recorded by Hoko automatic feeders.(* P < 0.05, **P < 0.01, *** P < 0.001)*

	Flight Speed	**Crush Score**	**Aggression Index**	**Displacement Index**
Lying Behaviour				
Total Daily Lying Duration	-0.03	0.17	-0.20	**-0.35
Mean Lying Bout	-0.10	-0.01	-0.04	0.05
Standing Behaviour				
Total Daily Standing Duration	0.02	-0.15	0.14	*0.27
Mean Standing Bout	-0.05	-0.15	0.09	*0.26
Activity Measures				
Step Count	**0.34	-0.07	0.09	0.01
MotionIndex	**0.35	-0.05	0.12	0.09
Measures of Variation				
S.D. Step Count	0.24	-0.04	-0.14	-0.11
S.D. Daily Lying Duration	-0.19	-0.09	-0.19	-0.13
S.D. Daily Standing Duration	0.01	-0.06	0.12	*0.27
Novel Activity Measures				
MotionIndex per standing bout	0.16	0.05	0.08	-0.03
Step Count per standing bout	0.23	-0.04	-0.01	0.06
MotionIndex per minute standing	*0.29	0.01	0.10	0.00
Step Count per minute standing	*0.27	-0.01	-0.06	-0.23
Feeding Behaviour				
Mean daily feeding duration	-0.16	-0.07	-0.08	0.07
Mean Kg consumed per day	*-0.24	-0.21	0.15	**0.36

4.4.3.2 Displacement index and aggression index

Displacement index had a positive correlation with average standing bout duration (r_s = 0.26, P = 0.036), the standard deviation of daily standing time (r_s = 0.27, P = 0.030), and the average daily standing time (r_s = 0.27, P = 0.028). Unsurprisingly, this meant the relationship between displacement index and daily lying time was negative (r_s = -0.35, P = 0.004). This suggests that dominant steers stood for longer on average and were more variable in their daily standing time. There were no significant correlations between aggression index and home pen activity ($P > 0.05$). Displacement index had a positive correlation with the average daily kg consumed from the Hoko bins (r_s = 0.36, P = 0.002), Aggression index did not have a relationship with average daily kg consumed ($P > 0.05$). Neither displacement index nor aggression index had any relationship with the daily average time spent feeding ($P > 0.05$).

4.5 Discussion

The primary aim of this study was to describe the extent of the relationship between accepted temperament assessments and home pen activity in beef steers using remote sensors. As the use of remote sensors in this area is still new the study also characterized steer activity and investigated what kind of activity measures may be useful to animal scientists.

In this study, steers which showed a high flight speed response, assumed to have found the handling experience extremely aversive, were also more active in the home pen. This is shown by the higher MotionIndex and average daily step count recorded over a period of weeks in the home pen. They were also more variable in their step count, as shown with the positive relationship between flight speed and the standard deviation of step count. The standing bouts of steers with a fast flight speed were not more active in general (shown by the non-significant relationship between steps per standing bout and flight speed), so it is not that higher levels of activity are evenly distributed throughout the standing bouts. Instead, these steers must have a few highly active bouts. It could be speculated that this is the steer reacting to some fear eliciting stimulus, while its calmer companions do not. Crush score, measured only minutes before the flight speed, did not show any relationship with activity measures. This may imply that, although flight speed and crush score are correlated, they are affected by overlapping underlying components, rather than the same specific component. It is possible that crush score is a measure of the steer's reaction to handling and flight speed is a more general measure of fearfulness. This continues the discussion in the literature questioning the sensitivity of crush score when subjective scores may miss out subtle differences in behaviour (Stookey et al. 1994;

Baker et al. 2003; Curley et al. 2006). Alternatively, steers with a high flight speed may simply be more active overall due to some intervening explanatory variable such as their agility or fitness and fearfulness may not be the underlying measurement. Regardless, this paper does not aim to address the non-unitary characteristics of temperament (Petherick et al. 2009a; Petherick et al. 2009b), but to propose that in this system flight speed bears a relationship with home pen behaviour and crush score does not. Note also that the flight speed and crush score had no relationship with aggression index or displacement index, suggesting some discriminant validity between these tests (Waiblinger et al. 2006). Steers which were consistently capable of displacing other steers from the feeders (i.e. had a high displacement index) had a lower daily lying time and a longer average standing bout duration. They were more variable in their standing time (as shown by the positive relationship with their standard deviation of average daily standing time). The aggression index did not show any significant relationships with activity. Aggression may be thought of as one tool which an animal can use to obtain a resource and, as demonstrated by the correlation between aggression index and displacement index, it is a method used commonly by those steers which often gain access to the feeders. From these results it appears to be the ability to displace rather than the aggression shown which impacts on behaviour in the home pen. However, aggression was only measured in one context, at the feeders, and over a relatively short period of time. The aggression index equation used in this study does not capture all forms of aggression and may instead be thought of as a steer's propensity to engage in an aggressive interaction at the feeder. A measure of aggression which incorporates more aggressive instances may find a relationship with home pen activity, but most

measures of aggression are confounded with resource competition and therefore dominance. It may seem counterintuitive at first to find that steers more capable of displacing others from the feeders spent less time lying than less-dominant steers, but these steers also consumed more kg of food per day. It may be that these steers were simply hungrier and thus more motivated to displace others. It is important to note that these steers were not expected to compete for resources to the point of exclusion. Food was provided for all steers and those which could not adapt to the feeders were removed from the study. They were fed *ad libitum* and with a good steer:feed bin ratio. Social dominance theory proposes that in social animals, dominant individuals are not limited behaviourally, whereas subordinate individuals must fit their behaviours around dominant group members (Deag 1977). A dominant steer would then be able to engage in any activity it wishes, whereas a subordinate steer might have to interrupt its preferred activity to take advantage of an unoccupied resource such as a feeder. However, high dominance and high levels of stress have been found to correlate in wild animals (Creel 2001). In domesticated situations where resources are not limited, an ability to displace other animals may demand that an individual spends a lot of time standing.

Temperament in animals is thought to be a general underlying trait mediating response to stimuli (Lyons et al. 1988). Some question exists as to whether this is a domain general trait which affects responses to many stimuli, or a contextual trait (Réale et al. 2000). If temperament is a general trait we would expect a steer which performs in an aggressive manner in a temperament assessment to also respond aggressively to stimuli encountered in its day-to-day lives, i.e. responding to a

conspecific's proximity. Whereas, if these traits are more specific, the assessments would bear little relevance to the steers day-to-day activity. This study contributes to the evidence suggesting that temperament is a general trait, as we already know temperament can affect productivity. Flight speed scores are often associated with low but significant correlations with production traits such as daily gain and meat eating quality (Voisinet et al. 1997a; Müller & von Keyserlingk 2006; Sant'Anna et al. 2012). Some of these studies have also found genetic correlations between flight speed and production traits, raising the possibility of breeding for temperaments more suited for production. The mechanisms by which temperament can affect production are not well understood. (Cafe et al. 2011) found Brahman steers with higher flight speeds also had decreased feed intake and spent less time eating, although in the same experiment, the same relationships were weaker in Angus steers. We have found a similar relationship between flight speed and feed intake, but by incorporating day-to-day home environment activity data, we can also attribute a higher level of activity to steers with high flight speeds. By utilizing activity measures in future, it may help to shed more light on the generality of temperament traits. (Van Reenen et al. 2005) suggests that temperament has an 'activity' component, that is to say that in response to any stimuli, some animals will be 'passive copers' and exhibit very little behavioural reaction relative to the level of internal stress they are experiencing. It may be that the temperament tests in this study, as they depend on observing and quantifying active behavioural reactions, are unable to identify passive copers. We would not expect a passive coping steer to be passive in temperament testing but suddenly active in its home environment. It is possible that both the activity and temperament measures in this study can only

identify animals which have an active behavioural response to stimuli. With this caveat in mind, we can be confident that the temperament tests in this study are measuring a consistent underlying trait as they have produced similar repeatability estimates to those seen elsewhere (Kadel et al. 2006; Kilgour et al. 2006; Hoppe et al. 2008; Gibbons et al. 2009b). The repeatability of activity in the home pen has not been explored in great detail in the literature, however, some measures of activity such as general locomotion levels in dairy cows have seen good repeatabilities (Schrader 2002; Müller & Schrader 2005b) The use of the bout calculation method developed by Tolkamp et al. (2010) should be encouraged by the good repeatabilities generated in this current study and suggests that activity too is consistent across time. As such, the relationships seen between day-to-day activity in the home environment and temperament in this study lend a great deal of weight to the argument suggesting temperament is a general trait, goes on to affect the lives and welfare of cattle and should be considered in production studies.

4.6 Conclusions

By examining how temperament affects home pen behaviour, this study has established that there can be a cost to some temperament traits such as displacement capability, even when feed is provided *ad libitum* and the ratio of steers to feeders is generous. The link between the flight speed response and the daily number of steps taken, combined with a lower feed intake suggests a possible mechanism for the link between flight speed and production traits such as average daily gain. Incorporating day-to-day activity in studies linking temperament and production traits may provide

a more thorough understanding of how individual variation in behaviour affects both production and animal welfare in different management systems.

Chapter Five

Fear responses to novelty in testing environments are related to day-to-day activity in the home environment in dairy cattle. [4]

[4] For this chapter I was responsible for: experimental design, data collection, data analysis, write up of the chapter.

With thanks to the staff at Wageningen's Dairy Research Centre for their assistance setting up the testing area and moving cows prior to testing. I am particularly grateful to Jan Zonderland and Joop van der Werf for assisting me in the logistics of carrying out the experiment daily on the farm. Tamara Wind, a final year undergraduate from CAH Dronten University was invaluable for her assistance throughout this experiment and her ability to translate. Ian Nevison from BIOSS provided statistical consultation for the multiple regression work.

5.1 Abstract

Fear in dairy cattle can be a welfare challenge and is often associated negatively with production traits. In dairy cattle, a novel arena-novel object (NANO) test is thought to test the cow's fear of novelty and a human approach (HAP) test is thought to test the cow's fear of humans. These kinds of tests take time to perform and can be stressful for the animals but are currently the only way of assessing behavioural reactions to fear-causing stimuli in a standardised manner. It would be advantageous, therefore, to be able to identify fearful animals using behavioural data collected remotely in the home pen environment through the use of activity monitors and robotic milkers. In this study we investigated the relationship between home pen activity measured by biotelemetry systems and behaviours recorded in both HAP and NANO tests. Eighty five dairy cows were NANO tested and 79 of these were also HAP tested. All animals had their activity recorded for 40 days prior to the test period using a tri-axial accelerometer biotelemetry system. High numbers of novel object contacts in the test was associated with younger animals with fewer lying bouts per day and were less variable in their lying bout duration ($R^2_{adj} = 0.13$, $F_{3,75} = 4.65$, $P = 0.005$). Cows which had a higher tolerance for human approach had fewer lying bouts per day, a shorter average standing bout duration and presented themselves to the robot milker more often ($R^2_{adj} = 0.08$, $F_{3,69} = 3.12$, $P = 0.032$). Personality traits constructed from a Principle Components Analysis of the observed NANO behaviours were also associated with home pen activity. Cows which scored highly on the first component termed 'neophobia' were older, had more lying bouts and a greater variation in the duration of their average lying bout ($R^2_{adj} = 0.15$, $F_{3,75} = 5.32$, $P = 0.002$) while cows which scored highly on the second component termed

'boldness' were older cows with less variation in their average lying bout (R^2_{adj} = 0.11, $F_{2,75}$ = 5.63, P = 0.005). To conclude, significant relationships exist between behaviours in short-term personality tests and home pen activity recorded by pedometers over several weeks. As fearfulness is reflected in spontaneous home pen behaviours, activity databases could be incorporated into models predicting fearfulness and welfare assessment protocols.

5.2 Introduction

A predisposition for an individual to have a fearful response to a stimulus repeatable across situations has been seen in many species. This was reviewed by Boissy (1995), has been seen in non-chordates such as squid (Sinn et al. 2008); chordates such as fish (Bell et al. 2009b); birds meant for re-release into the wild (De Azevedo & Young 2006); farmed mammals such as mink (Malmkvist & Hansen 2002), pigs (Andersen et al. 2000) and cattle (Gibbons et al. 2009a), horses (Malmkvist & Christensen 2007); and companion animals such as dogs (Svartberg 2005). 'Fearfulness' can be termed a personality trait (Uher 2011), which is to say it is an underlying component driving variation in the animal's behaviour and is to some extent repeatable and consistent across time and situations. This dimension, sometimes referred to as the shy-bold continuum in the five factor personality model (Gosling & John 1999), explains some of the behavioural variation observed in a population's reaction to a stimulus. Similar stimuli provoke similar responses, for example, reactions to a novel arena will be similar to reactions to a novel object in cattle (Boissy & Bouissou 1995) and both have been considered expressions of neophobia. However, fear expressed towards humans is not the same as fear

expressed towards novel objects (Boissy & Bouissou 1988) therefore these fear tests have been shown to have convergent and discriminant validation (Waiblinger et al. 2006). The role of fear in the behavioural repertoire of domesticated species and their interactions with their environment and conspecifics is a complex topic but one which has been intensively studied in dairy cattle. Originating from prey-animals, dairy cattle have a capacity for fear which alters their behavioural responses to stimuli and as such, fearfulness is an important trait for them, often negatively impacting their welfare (Von Keyserlingk et al. 2009). Humans may be regarded as potential predators even in their role of stock people and can be a source of fear for domesticated cattle (Hemsworth 2003) often limiting milk production (Breuer et al. 2000). This can be moderated by the quality of stock person behaviour. So-called 'friendly' interactions with stockpeople can lower the herd's median avoidance distance (Waiblinger et al. 2003), thought to be an indicator of how wary the cows are about her handlers. A state of fear can be brought about by other stimuli, even stimuli regularly encountered in the home environment. Forkman et al. (2007) noted that a fear-eliciting event can be characterised by its novelty or, conversely, through negative associations with a previously experienced event. Similarly, social events between conspecifics can also cause fear, such as in regroupings which force dairy cows to re-establish dominance hierarchies with agonistic interactions as these agonistic interactions may cause fear in low ranking animals (Raussi et al. 2005). Dairy cattle may therefore encounter fear-provoking stimuli in their home pen environment. Although there are many behavioural tests which attempt to quantify the fear experienced by cattle, these tests only record behaviour during the test, which is typically undertaken outside of the home environment. We can only infer

from this how the personality trait of fearfulness might affect the cow's behaviours in its day-to-day life in the home environment. Recent developments in remote sensors have enabled ethologists to collect data over long periods with little affect on the observed behaviour (MacKay et al. 2012). In this study we hypothesised that dairy cattle activity in the home pen would bear some relationship with fearfulness as measured in a combined novel arena and novel object (NANO) test and in a human approach (HAP) test. In the future, if fearfulness could be assessed remotely, this would enable on-farm welfare assessments to be done quickly and without labour-intensive testing of individual cattle. Accelerometer based activity recording is becoming more popular on farms due to their ability to detect oestrus events in cattle (Firk et al. 2002) with data being stored continuously in most systems, creating large databases of cattle behaviour data. If these can be utilised to identify animals with differing levels of fearfulness it could enable remote welfare assessment, or quicker selection for breeding programmes. Before this is possible, the relationship between personality and day-to-day activity must be more clearly understood.

Previous studies have related temperament scores, as proxy measures of fear, to activity in the home pen. Flight speed from a crush, a proxy of reaction to handling, has been found to relate to activity in the home pen in beef steers (chapter three). In dairy cattle, kick and step-counts during a tail-fixing test have been negatively correlated with the duration of lying periods in the home pen (Schrader 2002). These tests are context specific and quantify behaviours which are thought to be driven by fear, e.g. the assumption is that a fearful cow will be faster in a flight speed test. Another method is to construct personality traits from many observed behaviours in a

situation using multivariate analyses in a manner similar to Van Reenen et al. (2004). This construct approach, although still not measuring fear or similar traits directly, is thought to characterise fear more fully. The NANO test is frequently used in cattle fearfulness studies and the traits extracted via Principle Components Analysis typically relate to fear, as judged by the behaviours they were constructed from (Van Reenen et al. 2002; Van Reenen et al. 2005). The human approach test measures fear of humans and is most repeatable over time when done in the passageway in the animal's home environment (Gibbons et al. 2009a). This is a different aspect of fear (fear of humans as opposed to fear of novelty) and so is analysed separately. In this study, we chose to relate home pen activity not only to the behaviours observed during testing, but also to the traits constructed from observed NANO behaviours.

To record the cows' activity in the home pen we used tri-axial accelerometer type data loggers which attach to the cow's hind leg and sample the orientation of the device at a frequency of 4Hz. This kind of sensor has been found to be both accurate and specific (Tolkamp et al. 2010; Nielsen et al. 2010) and to have little effect on the cow's behaviour (MacKay et al. 2012), making them an ideal device for long-term monitoring and remote collection of data. In this study, the aim was to use activity data prior to the fearfulness test to predict the behavioural response of dairy cattle to novelty and humans.

5.3 Material and methods

5.3.1 Animals, housing and management

The Wageningen University Dairy Research Centre (Goutum, the Netherlands) has a milking herd of over 100 Holstein-Friesian dairy cows. The animals are housed indoors all year round in two separate groups, MS1 and MS2. There were no systemic differences between MS1 and MS2, they are intended to be at the same lactation stage, calving period and age. The division between the groups is for logistical reasons on the farm. Primiparous heifers enter either groups MS1 or MS2 depending on which group has space at the time. Cows exit the groups prior to calving and are re-introduced to the same group after calving, so no animal moves between MS1 and MS2. Each group was fed total mixed ration *ad libitum* and milked via a single robotic milker (DeLaval Industries) for each group. All animals had access to concentrate feed at automatic dispensing buckets and a grooming brush. During the bulk of the data gathering period, between February-May of 2011, monthly censuses of the herd estimated the average group size to be n = 58 ± 3 (S.D.) for MS1 and n = 46 ± 1 animals in MS2. A third group is housed within the barn which was not involved in the current study and did not mix with the study animals at any point.

In the spring of 2011 the animals were subjected to three forms of a personality test. The three tests were a social motivation test (SOC), a human approach test (HAP) and combined novel arena and novel object test (NANO). All NANO tests were carried out in the afternoon (PM) period and SOC tests in the morning (AM) period (**Table 5.1**). The tests were also staggered so MS1 received their NANO tests during the same period that MS2 received their SOC tests and vice versa. HAP tests were

carried out on days where no other testing was done on the group after the relevant SOC test had been carried out. This study focuses on the NANO and HAP tests, thought to reflect fear of novelty and fear of humans. Sociability results are not presented.

Table 5.1 *Structure of 40 days activity monitoring and Novel Arena/Novel Object (NANO), Sociability (SOC) and Human Approach (HAP) testing over 69 day test period for groups MS1 and MS2*

	Phase 1		**Phase 2**	
MS1	Days 1-14 NANO1	Days 15-29 NANO 2	Days 43-54 SOC 1 (Days 43-50) HAP (Days 53- 54)	Days 55-69 SOC 2 (Days 58-65) HAP (Days 68-69)
MS2	SOC 1 (Days 1-9) HAP (Days 11-12)	SOC 2 Days (15-23) HAP (Days 25-26)	NANO1	NANO 2

5.3.2 Novel Arena/Novel Object (NANO) testing

The combined Novel Arena/Novel Object (NANO) test was based on methodology established by Van Reenen et al. (2004) and adapted to form the combined test as follows. All animals within MS1 and MS2 were initially considered eligible for testing. The aim was for all animals to be tested twice, but some animals left the herd for calving or management culling and so were not available for second testing. The novel arena was a portable, covered shed measuring 5m by 5m fitted with a microphone and a CCTV camera in the roof-space allowing for a full view of the arena. Entry was via a manually operated door at the middle of one wall of the arena. Access to the door was through the start box, made of the same material and to the same height of the arena's walls. The cows could not turn around once in the start box and were prevented from backing out by a metal bar that swung down once the cow had entered the start box. The arena was erected outside the animals' home pen.

It was not visible to the animals outside of testing hours as the doors were closed and during testing was only visible when the animal stood immediately behind the doors prior to the beginning of their test. When an animal was selected for testing it was removed from the home pen and herded through a 4m gated runway by the observer and a helper to the start box where it was confined for 3 minutes. After this time had elapsed, the doors to the arena were opened and the animal was tapped on the hindquarters by the helper to encourage it to enter the arena. Testing began when the doors were fully opened. The cow was then allowed to freely roam within the arena for 5 minutes. Behaviours were recorded (see Table 5.2) by an observer standing quietly outside of the arena (see **Table 5.2**), monitoring the animal on a video screen. Scoring was done live. When in the arena, the animal could not see any other animals, the yard, the observer or the helper. After the 5 minute novel arena period had elapsed, the observer gave a visual cue to a helper (not visible to the animal) who lowered the novel object down into the centre of the arena. The novel object was a blue plastic jerry can with a child's tambourine attached to its top. The object was rigged up on a pulley system and was suspended 6m high in the roof-space at the start of each test. It was judged to be unfamiliar to the animals as it was not used within the home pen by the farm staff and the tambourine made a noise not normally associated with such an object. The object was lowered to the floor to make a noise and then raised to a height of 1m above the ground. The correct height was marked on the rope to ensure standardisation between subjects. Prior to the object test, if the animal was standing under the area the can would be lowered into, verbal encouragement was given to move the animal. If the animal would not move within two minutes, the can was slowly lowered to prompt the animal to move out of the

way. These 'waiting' minutes were removed from the analysis. The object section of the NANO test lasted for 10 minutes, after which the animal was returned to the home pen, the walls were wiped down by a wet, clean cloth and the floor scraped and cleaned before the next animal was tested. At the end of each test day the arena was pressure washed.

Table 5.2 *Definition of behavioural measures and events recorded in cows during combined NANO test.*

Measures	Definition
Recorded during arena and object stage	
Standing duration (seconds)	Animal remains stationary with weight on all four legs, or on three legs with one raised or bent. Animal may move head.
Locomotion duration (seconds)	Animal moves all four legs to cover distance.[1]
Shifting (Count)	Animal remains stationary but all four legs move and head swings. Does not last longer than 4 seconds
Vocalisations (Count)	All types of vocalisation
Defecation/Urination (Count)	Animal defecates or urinates within the arena.
Contact with wall (Count)	Sniffing, touching or rubbing of the wall with the nose, tongue, head or shoulders
Contact with floor (Count)	Sniffing, touching or rubbing of the wall with the nose, tongue, head or shoulders.
Recorded during object stage only	
Latency to touch novel object (seconds)[2]	Time from start of entrance to arena to first contact with object with nose or tongue.
Contact with novel object (Count)[2]	Animals spends time with nose, tongue, head or shoulders in contract with novel object.

[1] Not used in analysis as directly confounded with standing duration, i.e. animal was in one of the two states throughout test.

[2] Non-normally distributed, natural log used in later stages of analysis

The second test was administered 14 days ± 1.4 after the first test. The animals were tested in a pre-determined order to ensure they were tested at the latest possible stage of lactation which would still ensure they were tested twice before leaving the herd. All animals which would remain in the group for two tests were initially considered to be eligible for testing and an animal was considered to have completed testing if it

had undergone both repetitions of the NANO test, regardless of whether it then left the herd before the total test period for all animals was completed. If the cow presented as extremely lame on the day of testing, as judged by on a scale based on Manson & Leaver (1988), their test was delayed. As a result, continuously lame animals were not tested. One cow was also excluded from testing for exhibiting extreme stress related behaviours during the first test and repeated, violent escape attempts. This resulted in ninety five lactating dairy cows receiving both repeats of the NANO test at an average age of 4.09 years ± 1.5 at first test and at 162 DIM at first test ± 132. This was split as n = 52 animals in MS1 (age 4.26 years ± 1.45, DIM 154 ± 113) and n = 43 animals in MS2 (age 3.89 years ± 1.58, DIM 173 ± 152).

5.3.3 Human Approach Test (HAP)

HAP testing occurred in the afternoon period following a sociability test and was based on a protocol developed by Gibbons et al. (2009). All animals within the group were considered available for testing, however animals judged to be lame were removed from the dataset. A HAP test commenced when the focal cow was standing idle in the passageway of the housing area, with sufficient space to move away from the experimenter and with no more than two cows standing within a 1m radius of the focal animal. When this criteria was fulfilled, the experimenter approached the cow from a distance of greater than 3m in a standardised manner. The experimenter approached the focal cow using strides of approximately 1.0m, using the space between cubicles to gauge distance. After every step the experimenter remained motionless for 10 seconds to allow the cow to respond. The experimenter approached diagonally from the front towards the cow's neck, avoiding eye contact with the cow,

looking towards the feet of the cow and keeping arms and hands close to the body. Avoidance was recorded using a flight response score (**Table 5.3**) which was defined as the distance at which the cow responds by taking two or more steps in the opposite direction from the approaching experimenter. In some cases, cows may shuffle their feet or take a half step or even a full step in the opposite direction, and the experimenter would continue to approach. Cows were also allowed to turn their heads away from the experimenter and the experimenter would still continue. An important modification to the protocol from Gibbons et al. was that the tester was familiar to the animals as the experimenter who removed cows from the group for NANO and sociability testing. This may have resulted in some increased wariness of the experimenter.

Table 5.3 *Definition of flight response score for the HAP Test, adapted from Gibbons* et al *2009.*

Score	Behavioural Response
1	Animal retreats more than two steps away when experimenter is <3m but ≥2m away
2	Animal retreats more than two steps away when experimenter is <2m but ≥1m away
3	Animal retreats more than two steps away when experimenter is <1m but ≥0m away
4	Animal retreats more than two steps away when experiment is 0m away (e.g. beside animal's head)
5	Animals does not move away when experimenter is 0m away
6	Animal retreats more than two steps away when experimenter raises arm to touch animal's head/shoulder for 10s period
7	Animal retreats more than two steps away when experimenter touches animal's head/shoulder for 10s period
8	Animal retreats more than two steps away as experimenter moves hand to animal's body/rump for 10s period
9	Animal retreats more than two steps away as experimenter moves hand to animal's udder/legs for 10s period
10	Animal does not move away upon completion of test.

5.3.4 Collection of activity and milking behaviour data

One hundred animals distributed between the two groups are fitted with commercially available tri-axial accelerometer based activity monitors, often referred

to as pedometers. The type of activity monitor used in this study was the IceQube™ (IceRobotics, Ltd. South Queensferry, UK). The IceQube samples the force of gravity on each accelerometer at a frequency of 4Hz and interprets this as being in a lying or standing position and, if standing, how many steps are taken within a given period. The device also records MotionIndex™, which is the summed acceleration at fifteen minute intervals. The step count is calculated from this variable, but MotionIndex is recorded at all times, whereas step count is only calculated when the tag is considered to be in a standing position. A transceiver in the milking robot downloads the information stored on the tag when the animal passes through the milking station. Thus, the tags are frequently downloaded and the animals have their activity continuously recorded. Typically studies which use activity monitors examine the data and use a criteria established by Tolkamp et al. (2010) to eliminate false positive lying bouts. However, the volume of data in this instance made this approach impractical. As such, rigorous exclusion standards were applied to the data gathered from the milking parlour transceiver to exclude possible false positive lying bouts. Any days which were judged to have incomplete data (fewer than 80 data points) were excluded. The following rules were established based on prior experience with the biotelemetry generated by IceTag systems (Chapter Four, MacKay et al. 2012) and by examining the existing dataset for normal distributions. Any lying times of < 200 minutes or > 1050 minutes were excluded, as were any records which recorded < 5 or > 16 lying bouts in a day. Any records with > 4000 steps or a MotionIndex value of > 20,000 had their step or Motion Index value deleted (often both as these were highly correlated due to the way step count is calculated), however the rest of the record remained in the dataset.

The robotic milking parlour in each group recorded the number of times each cow entered the parlour and was successfully milked, the number of times each cow entered the parlour and was refused a milking session (i.e. for having been milked too recently) and the yield (kg) of each milking session. The parlours were at the south end of each home pen. To access the parlour, a cow had to pass through a one-way gate leading to a waiting area. The cow could exit the waiting area either through the parlour or through a second one-way gate, both of which exited to the feedface. If a cow did not present for milking within a given time period the robotic milker would alert stockpeople who would then drive the cow towards the parlour.

The behavioural testing (NANO, HAP and SOC) occurred during a 62 day period between the start of March and the start of May, 2011. We were interested to know if activity could predict the results of the behavioural testing therefore activity data was collected over the 40 day period prior to the start of testing. Previous work had found relationships with 28 day periods (Chapter Four) of activity and preliminary analyses showed that the 40 days prior to testing showed similar activity within both groups and was consistent within the groups, that is to say no obvious disturbances of activity were seen. Any days with oestrus detected were removed from the dataset. Of the 95 animals with NANO test data, 84 also had activity data collected by the IceQube. The cows had an average activity period of 23 days ± 16. Of these, 71 animals also had HAP scores. A full list of activity traits is described in **Table 5.4.**

Table 5.4 *Description of activity and milking behaviour traits in the home pen in the prior 40 days to the behavioural testing period, recorded by the IceQube system and the DeLaval robotic milker.*
**MotionIndex defined in text*
†Excluded from predictive models

Activity Trait	**Description**
Mean Daily Lie and Standard Deviation † (minutes)	The animal's average total lying time in the pen in a day and the standard deviation of this over 40 days. Confounded with Mean Daily Stand.
Mean Lie Bout and Standard Deviation (minutes)	The average duration of a lying bout and the standard deviation of lying bouts over 40 days.
Mean Daily Stand and Standard Deviation (minutes)	The animal's average total standing time (standing periods also include locomotory activity) in the pen in a day and the standard deviation of this over 40 days.
Mean Stand Bout and Standard Deviation (minutes)	The average duration of a standing bout (standing bouts also include locomotory activity) and the standard deviation of standing bouts over 40 days.
Mean Daily Number of Lying Bouts (DNLB) and Standard Deviation † (Count)	Average number of lying bouts observed in a day and the standard deviation of this over 40 days. Confounded with Mean DNSB.
Mean Daily Number of Standing Bouts (DNSB) and Standard Deviation (Count)	Average number of standing bouts observed in a day and the standard deviation of this over 40 days.
Mean DailySteps and Standard Deviation † (Count)	Average number of steps recorded (in days where steps were not >4000) and the standard deviation of this over 40 days. Calculated from MotionIndex when tag considered to be in standing position.
Mean DailyMI and Standard Deviation	Average Daily Motion Index* (in days where DMI was not >20,000) and the standard deviation of this over 40 days.
Milking Behaviour	**Description**
Mean Milks/Day	The average number of successful milking sessions in the robot parlour per day
Mean Refusals/Day	The average number of refused milking sessions in the robot parlour per day
Mean Milk Yield/Bout (Kg)	The average kg yield of milk in a successful milking bout in the robot parlour.

5.3.5 Statistical analyses

5.3.5.1 Constructing personality traits from Principle Components

In the NANO test, 8 behaviours from the ethogram (**Table 5.2**) were utilised in the analysis. In order to extract a personality trait, these 8 behaviours were analysed with a Principle Components Analysis (PCA). A repeatability calculation based on a REML calculation described in Lessells & Boag (1987) on the observed NANO behaviours found them to be repeatable between the two tests (**Table 5.5**), with the exception of latency to contact novel object which only had a repeatability of 0.09. The repeatability between the two HAP tests is also shown. The NANO test is primarily a test of the animal's behavioural reaction to novelty and as such, some of variation between the tests could have resulted from habituation to the novel test (Erhard et al. 2006; Gibbons et al. 2009a). For example the latency to contact the object increased by over a minute between tests and there were fewer object contacts, defecation and urinations, and more time spent standing in the second test. It was decided to use the behaviours recorded in the first NANO test as a truer measure of the animal's reaction to novelty and to account for any effects of habituation, despite the good repeatability between tests of most of the behavioural traits. The PCA of the 8 NANO behaviours was run in GenStat (version 14). This PCA was based on a correlation matrix which standardises each variate through subtracting its mean and dividing by its standard deviation (Harding & Payne 2011), which allowed for the variates to be both counts and measurements of time. The resultant loadings were orthogonally rotated (see results) and the first three components were preserved based on a scree plot of their eigenvalues and the percentage variation explained (Field 2005). For the HAP test, which is not reliant upon novelty, an average score

from the two tests is presented. The HAP score was not used in the PCA as it did not measure the same response and analysed separately instead.

Table 5.5 *Behavioural measures (mean ± s.e.m.) recorded in 95 dairy cattle during two combined novel arena/novel object tests and in 79 dairy cattle for two human approach test scores.*

	Test 1		Test 2		Repeatability
	Mean	**S.E.M.**	**Mean**	**S.E.M.**	
Duration standing (seconds)	674.0	11.06	742.0	11.55	0.37
Latency to contact object (seconds)	68.2	9.55	125.8	16.09	0.09
N floor contacts	7.9	0.50	7.2	0.50	0.49
N object contacts	4.0	0.27	2.6	0.27	0.24
N wall contacts	18.0	0.84	12.3	0.72	0.29
N def/urinations	2.3	0.17	1.5	0.14	0.37
N shifts	8.1	0.38	7.1	0.43	0.41
N vocals	25.4	2.16	20.5	2.01	0.74
Human Approach Test	2.8	0.21	2.8	0.22	0.59

5.3.5.2 Relating personality to home pen activity

We wanted to know if home pen activity could predict the results of the NANO and HAP tests. Not all the individual behaviours recorded for the NANO test (shown in **Table 5.2**) were informative of the cow's behavioural state by themselves. For example, the number of defecations and urinations, while useful to include in a multivariate analysis such as a PCA, is not informative as a single measurement and so not useful to predict with home pen behaviours. Therefore of the observed behaviours in the NANO test, we only used duration standing, latency to contact the novel object and number of novel object contacts in the following analysis. These three behaviours plus the individual cow's score on the three Principle Components constructed from the measures in **Table 5.2** and the HAP score were used as response variables in a multiple regression model in GenStat version 14 (VSN International, 2011). As latency to contact object and number of object contacts were

non-normally distributed, the log of both were used. Similarly a square root transformation of the HAP scores was used.

Due to the lack of variables suitable to be treated as random terms in a generalised linear model with restricted maximum likelihood (I. Nevison, pers. comm.), it was decided that a multiple regression model was most suitable. The explanatory variables included in the maximal model were the home pen behaviour traits recorded in **Table 5.4**. Step count (and standard deviation of step count) was excluded for being too highly correlated with MotionIndex, as was the number of standing bouts which was too similar to the number of lying bouts and total daily lie duration which was confounded with total daily standing duration. As well as these thirteen traits, the animal's age at first test, days in milk at first test and their group was included in the model. The most parsimonious model for each response variable was then selected using a stepwise method of residual means squares. Explanatory variables were removed from the maximal model when they failed to meet the test criterion of their variance ratio being > 4 (Harding & Payne 2011). The best models for predicting the behaviours observed and constructed from fearfulness tests are reported.

5.4 Results

5.4.1 Personality traits established in the NANO test

The first three components of variation extracted by the PCA accounted for 29%, 17% and 14% of the variation in response to the NANO behaviour test, cumulatively explaining 60% of the behavioural variation seen in the NANO test. The orthogonally rotated behavioural measures separated well onto PCs 1, 2 and 3. Animals scoring positively on PC1 had a high latency to contact the object and had fewer objects. This component was termed 'neophobia' (**Figure 5.1**). PC2 was characterised with a high number of vocalisations and few instances of contacting the floor with the nose (**Figure 5.2**). This was termed 'vocalisations'. PC3 was characterised by a long duration spent standing during the test and few wall contacts and defecation events. This was termed 'boldness'.

Figure 5.1 *Factor loadings on Principle Components 1 and 2, termed 'neophobia' and 'vocalisations' respectively. † Denotes a variable with natural log transformation*

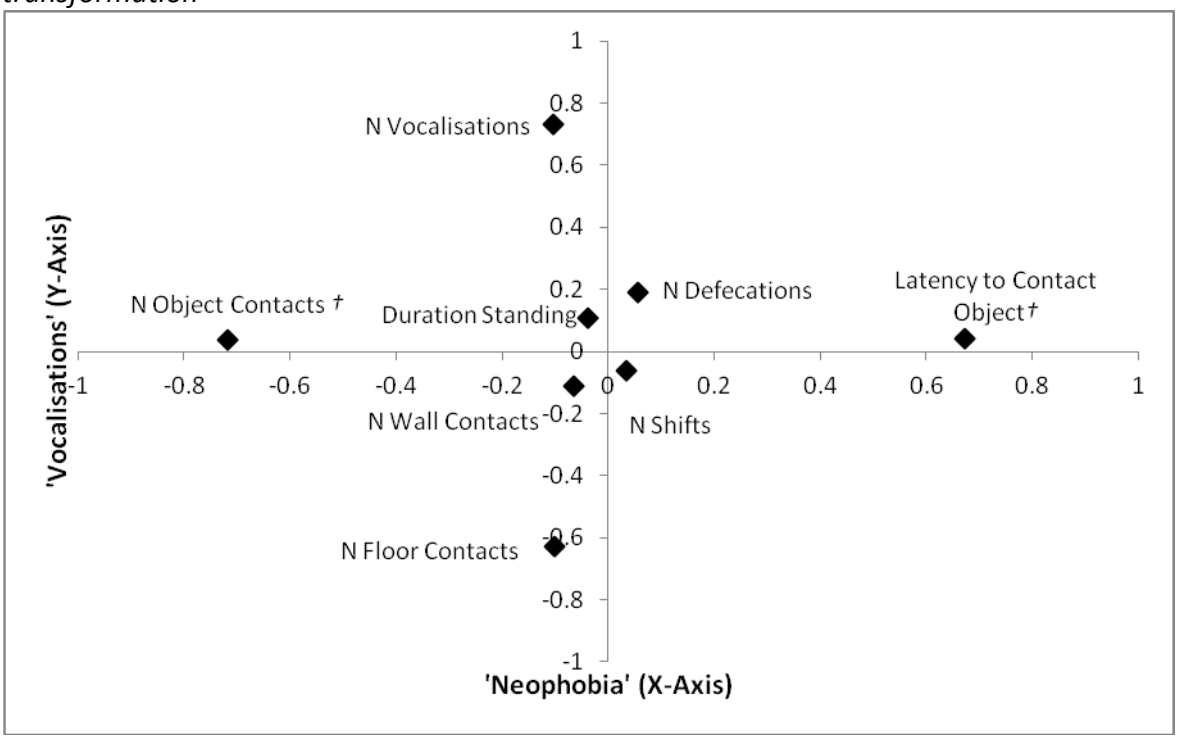
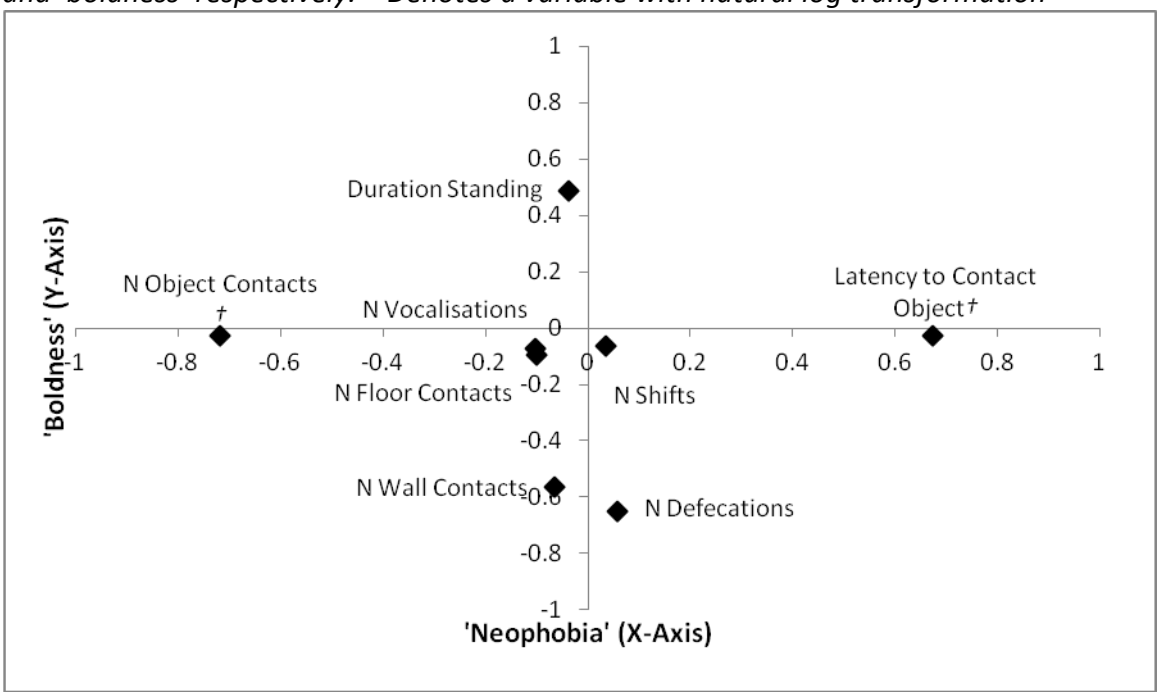


Figure 5.2 **Factor** *loadings on Principle Components 1 and 3, termed 'neophobia' and 'boldness' respectively. † Denotes a variable with natural log transformation*



5.4.2 The predictive power of home pen activity on HAP Scores

Cows which tolerated human approach (i.e. had a high HAP score) were more likely to have fewer lying bouts in a day, a shorter average standing bout duration and more milking bouts per day, however this model only explained around 8% of the variation in HAP responses. (R^2_{adj} = 0.08, $F_{3,69}$ = 3.12, p = 0.032; DNLB $t^{(66)}$ = -2.54, P = 0.014, average standing bout duration $t^{(66)}$ = -2.15, P = 0.036, average milks/day $t^{(66)}$ = 2.01, P = 0.049)

5.4.3 The predictive power of home pen activity on NANO observed behaviours

The latency to contact the novel object was most affected by the cow's days in milk at first testing and the mean yield of milk (Kg) per milking session (R^2_{adj} = 0.08, $F_{2,75}$ = 4.34, P = 0.017; days in milk at first test $t^{(73)}$ = 2.62, P = 0.011, mean milk yield (Kg) per milking session $t^{(73)}$ = 2.45, P = 0.017) with both being associated with an increase in the cow's latency to contact the novel object. The number of novel object contacts was associated with younger cows with few lying bouts recorded in the home pen and less variable lying bouts in the home pen (R^2_{adj} = 0.13, $F_{3,75}$ = 4.65, P = 0.005; age at first test $t^{(72)}$ = -2.60, P = 0.011, DNLB $t^{(72)}$ = -2.45, P = 0.017, standard deviation of average lying bout $t^{(72)}$ = -2.13, P = 0.036). Home pen activity and milking behaviour could not account for any of the variation seen in the time spent standing during the NANO test (P > 0.1).

5.4.4 The predictive power of home pen activity on NANO constructed traits

The constructed variable 'neophobia' was associated with more lying bouts per day, a greater variation in the duration of their average lying bout and were older (R^2_{adj} = 0.15, $F_{3,75}$ = 5.32, p = 0.002; age at first test $t^{(72)}$ = 2.89, P = 0.005, DNLB $t^{(72)}$ = 2.56, P = 0.012, standard deviation of average lying bout $t^{(72)}$ = 2.15, P = 0.035). 'Boldness' was associated with older cows and less variable average standing bout durations (R^2_{adj} = 0.11, $F_{2,75}$ = 5.63, P = 0.005; age at first test $t^{(73)}$ = 2.52, P = 0.014, standard deviation of average standing bout $t^{(73)}$ = -2.72, P = 0.008). 'Vocalisations' could not be predicted by home pen activity traits or milking behaviours (P > 0.1).

The direction and size of the associations between the predictive variables and their responses is shown in **Table 5.6**.

Table 5.6 *Direction of effect (+ve or –ve) of predictive variables on responses and percentage of variation explained by the model. (+/- P < 0.05, ++/-- P < 0.01)*

Response and predictive variables	**Effect Direction**	**Total variation explained by model**
Human Approach in the Passageway score		**8%**
DNLB	-	
Average standing bout duration	-	
Average number of milking bouts per day	-	
Latency to Contact Novel Object		**8%**
Days in milk at first testing	+	
Mean milk yield (Kg) per milking bout	+	
Number of novel object contacts		**13%**
Age at first test	-	
DNLB	-	
Standard deviation of average lying bout duration	-	
'Neophobia' (Principle Component 1)		**15%**
Age at first test	++	
DNLB	+	
Standard deviation of average lying bout duration	+	
'Boldness' (Principle Component 2)		**11%**
Age at first test	+	
Standard deviation of average standing bout duration	--	

5.5 Discussion

Novelty tests and response to humans have been used in cattle for welfare assessment purposes (Hemsworth et al. 1996; Windschnurer et al. 2008) and it has been speculated that reliable methods for the assessment of personality may enable these traits to be selected for (Gibbons et al. 2009). However, it is the duration and equipment required for such tests which make them difficult to adopt on a large scale (Waiblinger et al. 2003) as well as some uncertainty as to how these short tests relate to long term behaviour (Forkman et al. 2007). In this study we have established that home pen behaviours such as activity and trips to a robotic milking parlour may contribute to remote assessment of behavioural traits in dairy cattle. Prior to this study, the only way of establishing a dairy cow's response to novelty was to test her in an arena. Here we have shown that some of that variation can be predicted through easily gathered home pen traits. While this remains a small proportion of the variation (ranging between 15% of the variation in 'neophobia' and 8% of the variation in HAP score and latency to contact a novel object) it is the first step in being able to remotely identify fearful animals, paving the way for large scale activity database usage for welfare assessment.

5.5.1 The validity of short duration assessments

An important aspect of our results has been establishing that a relationship exists between the natural activity behaviours displayed in a home pen and the behaviour exhibited in short behavioural tests such as the HAP test (which is concluded in a matter of minutes) and the NANO test (which lasts for 15 minutes in an unnatural

testing arena). One of Forkman et al.'s (2007) criticisms of the fear tests used in farm animals was that they were rarely validated and that testing in inappropriate environments may result in inaccurate estimations of fear. In this study we were keen to use what could be considered as 'gold standard' tests, which had been used in a large range of systems and found to be very repeatable. The three components extracted from the NANO test closely reflected those seen previously by Van Reenen et al. (2004). In this study, the trait of 'neophobia' had significant associations with home pen behaviour, however 'vocalisations' did not and 'boldness' was most related to the cow's age. It is important to highlight the predictive power of age in the models predicting object related behaviours. Similar to previous work in beef cattle standard deviation as a measure of variability in the animals' activity pattern was also informative. High variability in activity traits may then be an indicator of an animal easily influenced by novel stimulus.

The contrast between the models for predicting the observed NANO behaviours and the constructed components of NANO variation is particularly reassuring for proponents of Principle Components analysis. Principle Components are, after all, statistical constructs and must always be used with caution. It is interesting to note that time spent standing in the NANO test has no significant associations with home pen activity whereas the component 'boldness' does. Time spent standing has a strong positive loading on the 'boldness' component (see **Figure 5.2**) and so this clearly illustrates where the constructed components, by incorporating more behaviour information, can more clearly reflect the trait which goes on to affect the animal's day-to-day life. Similarly, age and lying behaviour in the home pen could

predict up to 15% of the variation in 'neophobia' but only up to 13% in the number of novel object contacts which loads very highly on 'neophobia'. This result would also suggest that the NANO test itself is measuring something other than simply a general underlying activity level, which is a concern when using fearfulness tests which measure activity as an indicator of fear (Chapter Four). The lack of a relationship between home pen activity and 'vocalisations' is perhaps unsurprising as cattle are capable of vocalising when they are in a variety of states. That is not to say that vocalisations are unimportant in the study of fear, by the contrary cattle vocalisations carry a great deal of information (Watts & Stookey 2000), but how this information may relate to home pen activity is not immediately obvious.

Interpreting cattle behaviours in the context of fear is a large part of the fearful behavioural response literature. Sniffing and licking of a novel arena can be viewed as expressions of the cow's desire to explore and are affected by prior experience of an arena, locomotion and activity during the same tests has been seen as both fearfulness and an underlying motivation to be active (De Passillé et al. 1995). In such tests we wish to record all possible behaviours the animal might express, but reduce these to more manageable components of variation. Principle Components Analyses essentially arrange data in n-dimensional space and rotate this matrix to find the axes which explain the most variation in the dataset. For example, in similar novelty tests, extracted components of variation have been referred to as 'agitation' and 'human avoidance' in beef steers (Kilgour et al. 2006) which collectively explained ~40% of the variation in response to the novelty test. Similar components were referred to as 'locomotion', 'vocalisation', 'novel object interaction' and

'human interaction' in dairy heifers (Van Reenen et al. 2004) which collectively explained ~80% of variation in response to the novelty test. In horses they were referred to as 'flightiness' and 'sensitiveness' in horses (Visser et al. 2001) which collectively explained ~60% of the variation in response to the novelty test. These latent components are all statistical constructs explaining the behavioural variation and so are not measured in units that can be related to some underlying biology, but do explain the individual's variation in relation to the population and therefore can be termed personality traits. With the validation of day-to-day home environment activity patterns found in this present study we can be confident that behaviour displayed in the short term NANO test does reflect underlying traits which go on to affect the day-to-day lives of cattle.

5.5.2 Predicting fear through home environment behaviours

Although this study has found significant associations between home pen activity and behaviours in a test environment, more research is needed before home pen behaviours can be used to predict individual personality. There are two possible explanations for why these relationships are not stronger. The first is that the tests themselves may be flawed and not identifying fear in cattle accurately. In Forkman et al.'s (2007) review, they discussed the idea that behavioural testing may not identify animals having an extreme internal reaction but a small observable reaction. This problem is still present within our study and it may be that the low R^2 value of some of the associations is because the tests are not identifying fear accurately, and so resultantly, the relationships between fear and home pen activity are weak. However, animals with a small activity component (often termed passive copers (Koolhaas et

al. 1999; Van Reenen et al. 2005)) may not have a large observable reaction in the home pen either. In one study of human disturbance of turnstones, *Arenaria interpres*, unfed birds would tolerate greater disturbance when food resources were scarce (Beale & Monaghan 2004). The authors of that study raised the point that for some animals, there may be no choice and they must suffer fear without displaying a behavioural coping mechanism. This remains a consideration when assessing levels of fear in animals; however the cows in the present study were well cared for and provided adequate nutrition for continued milk production. Additionally, the NANO test recorded many behaviours in a very unfamiliar environment and the constructed components of behavioural variation have been (as noted above) observed several times in many different studies. This may be a case where the component 'vocalisations' is detecting fear-related distress which is not being displayed in activity behaviour and so cannot be observed by activity monitoring in the home pen.

An alternative explanation for the relatively small amount of variation explained is that in this herd, fear-eliciting stimuli are not frequently presented to the dairy cattle, resulting in only a small amount of the variation in day-to-day activity patterns being driven by fear. In a herd under different management conditions, with more frightening stimulus present, the relationship between these tests and home pen behaviours may be stronger. The other issue which arises here is why we should expect to see fear reflected in day-to-day activity budgets at all. A recent comprehensive review lists a large variety of factors influencing individual level time budgets (Marshall et al. 2012) including differences in metabolic rate, whether large animals with larger rumen get hungrier faster and require longer bouts of

ruminations; fluctuations in temperature affecting metabolic efficiency and comfort; sexual status and hormonal changes. This review also makes clear that to understand how social groups (such as cattle) behave, it is necessary to understand individual level behavioural variation. The home environment of a farm can present startling stimulus such as unexpected loud noises, human interactions, aggression from conspecifics etc. but how many of these do we expect cows to react to? The significant relationships seen between home pen behaviour and fearfulness in the current study may suggest that we still have some way to go before fully understanding how fear impacts the day-to-day lives of domesticated cattle, and how we may go about improving this.

5.6 Conclusions

The two fearfulness tests, the human approach and the novel arena/novel object test were shown to reflect underlying traits which also affect a dairy cow's spontaneous behaviour in the home pen. It is possible to predict some of the variation in response to these tests using activity behaviour gathered in the home pen prior to testing. These results further raise the possibility of using remote sensor data to identify animals which face welfare challenges without the need for laborious testing.

Chapter Six

Responses in a social isolation test are related to milk production and activity in the home environment in dairy cattle.[5]

[5] For this chapter I was responsible for: experimental design, data collection, data analysis, write up of the chapter.

With thanks to the staff at Wageningen's Dairy Research Centre for their assistance setting up the testing area and moving cows prior to testing. I am particularly grateful to Jan Zonderland and Joop van der Werf for assisting me in the logistics of carrying out the experiment daily on the farm. Tamara Wind, a final year undergraduate from CAH Dronten University was invaluable for her assistance throughout this experiment and her ability to translate. Ian Nevison from BIOSS provided statistical consultation for the multiple regression work.

6.1 Abstract

A commonly used test of social motivation is the 'runway test' or social motivation test (SOC). This involves separating the subject animal from a group of conspecifics and recording its latency to return to the group and the activities shown while returning. This test has been used successfully in dairy cattle and is thought to reflect the underlying personality trait of sociability. A sociable cow would return to the herd more quickly than a non-sociable cow. As with all on-farm tests, the social motivation test can be labour intensive and potentially stressful for cattle. By relating test performance to automated measures of behaviour in the home pen it may be possible to remotely assess sociability without laborious on-farm testing. In this chapter, 74 dairy cows were given two SOC tests with two weeks between tests. Each animal was separated from the herd with the herd confined at the opposite end of a 12m runway. The subject was released and behaviours and latency to rejoin the herd were recorded for a 5 minute period. The average latency to reach a 5m distance from the herd and the duration of time spent in close proximity to the herd were among the variables included in a Principle Components Analysis to create a component referred to as 'isolation activity'. Home pen activity and milk production were recorded for 40 days prior to the start of SOC testing. In a multiple regression, 'isolation activity' was associated with average milk yield per day with cows showing more activity having a lower yield and being at an earlier stage of lactation at first test (R^2_{adj} = 0.14, $F_{2,68}$ = 6.60, P = 0.002). Other measures of activity during the SOC test, such as the number of times the cow crossed the threshold line 5m from the herd was also related to home pen activity (R^2_{adj} = 0.19, $F_{4,68}$ = 5.08, P = 0.001) with cows which crossed the line many times having shorter standing bouts,

longer overall standing duration in a day, producing less milk per day and being less variable in the number of lying bouts they have in a day. While this study finds that SOC test behaviours relate to home pen behaviours, there are some criticisms to be made of the SOC methodology in this study and further work is needed to more fully understand the relationship between lactation status and the personality dimension of sociability.

6.2 Introduction

An animal's underlying motivation to prioritise certain behaviours leads to consistent individual differences in behaviour. This may be thought of as a personality trait (Uher 2008) affecting the individual's behaviour across situations and is repeatable over time (Bell et al. 2009a). Sociability, defined here as the tendency to associate with conspecifics, is a trait affecting the behaviour of group-living animals. Sociable individuals can have a desire for social contact (Gibson & Mann 2008) or a desire not to be solitary (Hamilton 1971; Müller & Bossley 2002), but the outcome is that they put their desire for social proximity above other needs. This can often be seen as a trade-off with other needs and which has commonly been seen with foraging activity (Väisänen & Jensen 2003). For example, Scottish Blackface ewes which have a lower average distance from the herd will delay their approach to a concentrated food resource in preference to remaining with the herd (Sibbald et al. 2006). Social interactions can have positive effects on individuals. Brahman steers which can see and interact with a familiar steer were more likely to approach and make more use of a food resource than those paired with unfamiliar steers (Patison et al. 2010). In dairy cattle, allogrooming can significantly reduce heart rate in the recipient, if not the actor, making allogrooming a way of giving reassurance to a

conspecific (Laister et al. 2011). Not fulfilling the desire for social proximity can have negative effects on the animal. Dairy cattle, when separated from their peers have shown an increase in heart rate, blood plasma cortisol and show behavioural indicators of stress such as struggling and increased vocalisations (Boissy & Le Neindre 1997). On the other end of the scale, non-sociable silver fox vixens, when housed communally, have poorer welfare than their sociable companions (Hovland et al. 2011). In group living animals, social motivation helps to form larger groups (Whitehead 2008). The role of the individual, particularly when it is a highly gregarious, can have an effect on the social structure and formation of the group (Godde et al. 2013). Sociability is a complex trait: the presence of conspecifics can be a positive boon to welfare for more sociable animals, but negatively affect less sociable animals. In order to more fully study sociability and its affect on cattle, it would be advantageous to have a method of assessing it in individuals quickly and easily.

The sociability personality dimension exists within cattle (Færevik et al. 2006), is consistent across at least two lactations (Müller & Schrader 2005a) and can be considered separate from other conspecific orientated behaviours such as aggression (Reinhardt & Reinhardt 1981). Within dairy cattle, the strong social bonds which can develop within a herd can cause welfare problems. The frequent regrouping of modern dairy herds is considered to be a potentially stressful process (Von Keyserlingk et al. 2008) with newer animals receiving more agonistic interactions, spending less time lying and using less of the available space (Gygax et al. 2009). Introducing groups of newer cows together does not necessarily induce them to form

new subgroups within the established herd (Neisen et al. 2009). Mixing stress can affect milk production and feeding durations (Hasegawa et al. 1997) and affect the animal's behaviour in other stress tests such as introduction to a dog and physical restraint (Boissy et al. 2001). Further to this, dairy cows do not appear to habituate to continued regrouping (Raussi et al. 2005). As more sociable animals respond differently to social stresses than less sociable animals (Müller & Schrader 2005a; Hovland et al. 2011) the dairy cow's own sociability helps to predict how she will respond to the stresses of living in a modern dairy system.

Sociability in dairy cows has previously been assessed using a response to social isolation test, often called a 'runway test' (Gibbons et al. 2010), where one animal is separated from the herd and their latency to return and general activity during the test is used as an indicator of how motivated the animal is to rejoin conspecifics. The traits in Gibbons' study were found to be highly repeatable (ranging from 0.39 to 0.54 for different test measures based on a within and between animal variance calculation) and easy to use on farm. The latency to reach a distance of 5m from the herd and other measures were associated with how closely a cow associated with her other cows in the home pen and how synchronous her behaviour was with the rest of the herd. The SOC test was designed with situational relevance, that is to say it occurs within the home pen and references behavioural response to social isolation. It has been validated by other tests referring to the trait of interest (Gibbons et al. 2010) and has ecological validity (as social motivation has often been linked to foraging trade-offs in sheep and deer (Sibbald et al. 2006; Bergvall et al. 2011)). Therefore the SOC test fulfils Carter et al's (2012a) criteria for selecting an appropriate test of a

personality trait. Gibbons et al also proposed that repeatable traits, such as sociability, may be used as a breeding criterion, if there could be some welfare advantage, i.e. breeding for cows which were not distressed by regrouping. Therefore, the SOC test is a good candidate for exploring the possibility of predicting personality variation through automated and remote measures of behaviour. It would be useful to compare sociability across farms and management systems but to do this is very labour intensive. If it could be done remotely via the widespread adoption of biotelemetry systems among farmers (Rushen 2012) then the impacts of sociability on the welfare of dairy cattle could be explored across a range of systems. Gathering large quantities of data is a task automated systems are particularly suited for. Furthermore, there is danger that these databases will not be fully utilised in welfare assessment if novel avenues are not explored (Rushen 2012). In this study, I related sociability, as measured using a social motivation test, to the home pen activity behaviour in 74 continually housed dairy cattle. Home pen activity was recorded by unobtrusive accelerometers (MacKay et al. 2012) providing an accurate record of some aspects of the animal's spontaneous, natural behaviour. Milking behaviour, such as number of visits to the milker and mean milk yield per milking bout were recorded by a robotic milking parlour. Activity and milking behaviour were recorded for the same 40 day period for all cows prior to the start of testing with the aim of using this period of remotely recorded behavioural data to predict the results of the SOC test. As considerable variation exists in dairy cattle activity patterns (Tolkamp et al. 2010), this study sought to understand whether this variation could be used to predict performance in the sociability test to enable the remote evaluation of cattle welfare.

6.3 Methods

6. 3.1 Animals, housing and management

The animals were managed as described in Chapter 4. This part of the study reports the results of the social motivation (SOC) tests which took place in the morning period (**Table 6.1**). Wageningen University's Dairy Research Centre (Goutum, the Netherlands) has a milking herd of over 100 Holstein-Friesian dairy cows. The animals are housed indoors all year round in two separate groups, MS1 and MS2 with no systemic differences between the groups. They are intended to be at the same lactation stage, calving period and age. Cows enter the herd depending on which of the two groups has space at the time, exit the herd when they are dried off for calving and return to the herd they left post calving. Both groups are fed total mixed ration *ad libitum* and milked via a single robotic milker (DeLaval Industries). The behavioural testing period was between February-May of 2011 when a monthly herd census estimated the average group size to be n = 58 ± 3 (S.D.) for MS1 and n = 46 ± 1 in MS2. During this study period, the cows were subjected to three forms of a personality test. The three tests were the social motivation test (SOC), a human approach test (HAP) and combined novel arena and novel object test (NANO). The SOC tests were carried out in the morning period with MS2 receiving their SOC tests during the first phase and MS1 received their SOC tests during the second phase (**Table 6.1**).

Table 6.1 *Structure of 40 days of activity monitoring and Novel Arena/Novel Object (NANO), Sociability (SOC) and Human Approach (HAP) testing over a 69 day test period for groups MS1 and MS2*

	Prior 40 Days	**Phase 1**		**Phase 2**	
	Days -40 - 0	Days 1-14	Days 15-29	Days 43-54	Days 55-69
MS1	Activity and milk traits recorded	NANO1	NANO 2	SOC 1 (Days 43-50) HAP (Days 53- 54)	SOC 2 (Days 58-65) HAP (Days 68-69)
MS2	Activity and milk traits recorded	SOC 1 (Days 1-9) HAP (Days 11-12)	SOC 2 Days (15-23) HAP (Days 25-26)	NANO1	NANO 2

6.3.2 Collection of activity and milking behaviour data

IceQubes (IceRobotics, Ltd., South Queensferry, UK) were fitted to 100 animals distributed between the two groups. The IceQube samples the force of gravity on each accelerometer at a frequency of 4Hz and interprets this as lying or standing behaviour and, if standing, how many steps are taken within a given period. The device also records MotionIndex, the summed acceleration at 15 minute intervals. A transceiver in the milking robot downloads the information stored on the tag whenever the cow passes through the milking station and thus tags are frequently downloaded, resulting in continuous recording of activity. The robotic milking parlour in each group records the number of times the cow enters the parlour and is successfully milked, the number of times the cow is refused a milking by the parlour (i.e. for having been milked too recently) and the yield (kg) of each milking session. For more information, please see Chapter Five. As in Chapter Five, both activity and milking behaviour data was taken for the 40 days prior to the start of the behavioural testing as this period of time was not interrupted by behavioural testing.

6.3.3 Social Motivation Test

6.3.3.1 Animals and selection criteria

All animals within MS1 and MS2 were subjected to a social motivation test if they were not expected to leave the herd (due to becoming dry) within the test period. As a result, 99 dairy cows successfully received both iterations of the SOC test. All animals were tested in the morning period between 9A.M. and noon. MS2 cows were tested from 28th February, 2011-24th March, 2011 and MS1 cows were tested from 10th April, 2011 – 4th May 2011 (**Table 6.1**). Each animal was tested as part of a test-group of 6 animals, with the test-group being balanced for age and lactation status. At the date of first test, the average days in milk was 168.8 ± S.D. 115.1 for MS1 (n = 56) and 164.2 ± 121.3 (n = 43) for MS2. Up to two test-groups could be tested in one day. Of these 99 cows, not all had had corresponding activity and milking behavioural data (see below) and 3 cows showed some oestrus behaviour in the three days immediately after the test and so were excluded from the models, making the final sample size n = 74.

Animals were allocated to a test-group to ensure that each animal was returning to a group of companion animals with a similar mix of ages and lactation stage, standardised as much as possible without handling the animals more than necessary. All 6 animals in the test-group were tested on the same day, with animals 1 through 6 being separated from the remaining 5 for their individual test. The rest of the herd were confined beyond the test-group companions (see Test Area below). In each group, MS1 and MS2, there were animals which were not fitted with activity monitors. They were included in the sociability testing to allow the largest number of animals as possible to be tested. Some of these cows were used in two test-groups to

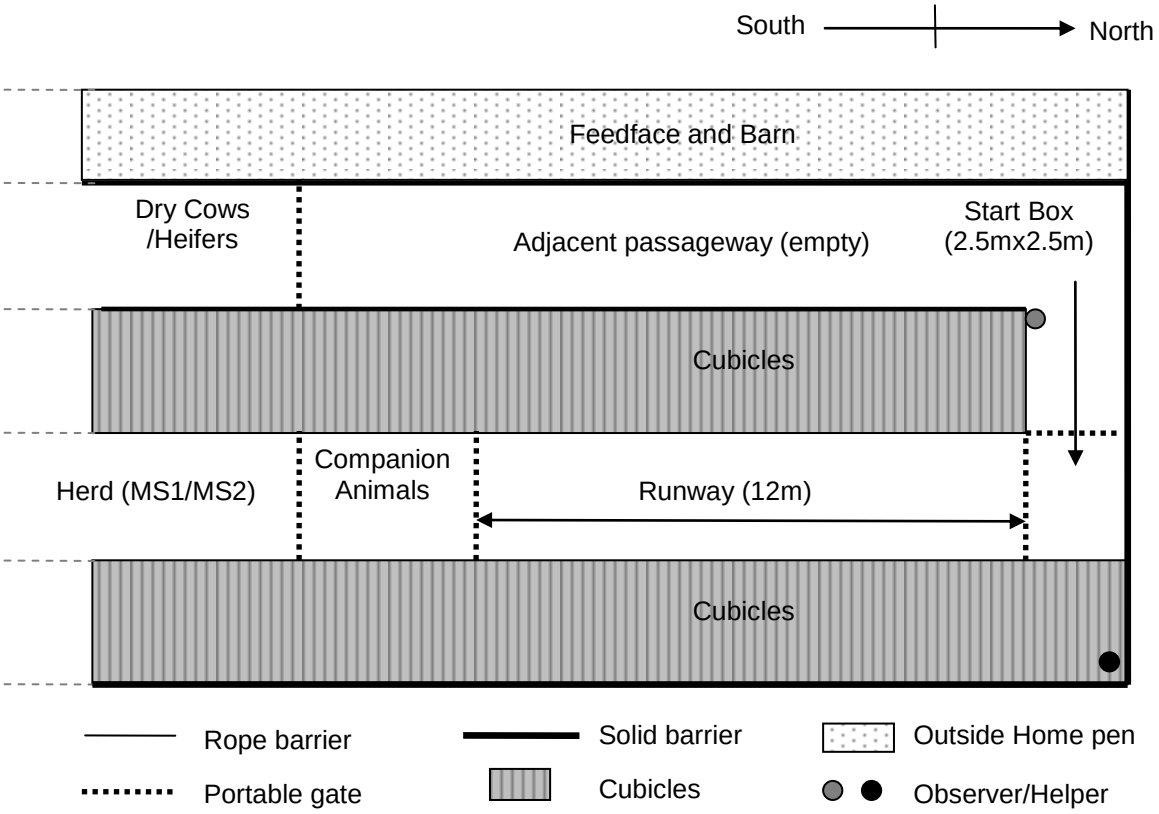
make up numbers for each group. A ‘spare’ cow would undergo her test in her true group and then the following week be a part of the ‘spare’ group where she would act as a companion animal only. Her repeat test then occurred in her true group the following week and a second spare test the week after. Spare animals did not have activity monitors attached so they contributed to the construction of the principle components only (MS2 n = 6, MS1 n = 2).

6.3.3.2 Test area

The SOC test was held in the home pen to reduce the levels of neophobia experienced by the animals (Waiblinger et al. 2006; Lansade et al. 2008). For each group, the test was held in the passageway between cubicles at the north end of the barn (**Figure 6.1**). During the test, access to the cubicles was barred by a thick rope strung taut between cubicle separators. A start box was created by a portable gate at the far north end of the passageway and was approximately 2.5m x 2.5m wide. The test runway extended along the passageway for 12m from the opening of the start box (start line). At the end of the runway another portable gate was erected across the passageway to pen in the test-group. Approximately 3.5m (3 cubicle widths) further along the passageway another portable gate was erected across the passageway to separate the companion animals from the rest of the herd. At this junction, in the adjacent passageway, another set of gates was erected to partition the herd from the dry cows (MS1) or heifers (MS2) which normally occupied the adjacent passageway. During runway tests, the passageway adjacent to the runway was empty. Each tested animal was returning to the five animals remaining in its test-group, to standardise the age and lactation stage composition of the companion animals. In the runway,

two lines were marked in sawdust at 2m and 5m from the companion animals and a chalk mark was made on the corresponding cubicle partition. From the start box, the subject animal could see the companion animals and behind the companion animals the rest of the herd. The subject animal could also see the experimenters who opened the start box, but the experimenters remained behind portable gate barriers throughout testing.

Figure 6.1 *Diagram of social motivation test area within MS1 home pen. MS2 test area is mirror image along the horizontal line.*



6.3.3.3 Test Procedure

Testing began at 9am when the herd was moved towards the south end of the barn (the end furthest from the test area) and penned in. The dry cows (MS1) or heifers (MS2) which occupied the area were moved down to the adjacent passageway by the

feedface and held there by a portable gate. The runway area was set up as in **Figure 6.1** and was scraped clean. The six animals in the test group were selected from the main herd and confined in the companion animal area. After confining all six animals in the companion area they were allowed ten minutes to settle. One at a time, the animals were selected for testing. The subject animal was removed from the companions quietly and taken to the start box. She was confined for one minute and then the start box was opened. If the animal had not moved when the gate was fully open she was given a tap on the rump. This was repeated after ten seconds if she had still not moved. No further motivation was given for the animal to leave the start box. The experimenters stood beside the start box and remained motionless for the duration of the test, however they could be seen by the subject and companion animals. Throughout the test, the subject animal's behaviour was recorded using a stopwatch and directly onto an Excel spreadsheet on a portable computer according to the definitions shown in **Table 6.2**. After five minutes, the test was over and the subject was returned to the companion animal group. The companions were given five minutes to settle while the runway was scraped and cleaned and the next subject animal was selected. When all testing had finished, the test equipment was dismantled and stored and the home pen returned to non-test conditions.

Table 6.2 *Definition of behavioural measures and events in SOC test*.

Measure or event	Definition
Test start	The animal's front hooves cross over the exit line of the holding pen.
Animal enters 5m and 2m lines (Count)	The animal's front hooves cross into the 5m from companion animals' box, and the 2m from companion animals' box.
Duration in 5m box and 2m box (minutes)	The animal's front hooves are present in the 5m box (time duration) and animal's front hooves are present in 2m box (time duration)
Duration outwith 5m Box (minutes)	The animal's front hooves no longer occupy 5m box and has moved away from companion animals (frequency and duration)
Vocalisation (count)	The animal makes any noise (type, duration and frequency)
Defecation/Urinates (count)	The animal defecates or urinates in passageway (frequency)

6.3.4 Statistical analyses

The repeatability of traits recorded in the SOC test were calculated as in Lessells & Boag (1987). The most repeatable behaviours observed in the SOC test were analysed via Principle Components Analysis (PCA) in GenStat (version 14). This PCA was based on a correlation matrix which standardises each variate by subtracting the variate mean and dividing by its standard deviation for each value (Harding & Payne 2011). This transformation allows for both counts and measurements of time to be compared in a single analysis. The resultant loadings were orthogonally rotated (see results) and the first three components explained 49%, 17% and 16% of the variation in SOC behaviour but only the first component was preserved based on a scree plot of their eigenvalues and examination of the components. Note that unlike the constructed traits relating to fear in Chapter 5, the

constructed sociability trait refers to behaviour averaged over both tests as the sociability test was not reliant on novelty.

As in Chapter 5, I wanted to know if home pen activity, including milking behaviours, could predict the results of the social motivation test. Due to the lack of variables suitable to be treated as random terms in a generalised linear model with restricted maximum likelihood (I. Nevison, pers. comm.), it was decided that a multiple regression model was most suitable. Therefore, the response variables were the constructed variable from the PCA and the most repeatable observed SOC behaviours: average number of times in the 5m box, average number of times in the 2m box, the average duration spent in 2m box and average latency to the 5m box, the latter two variables being transformed with a natural logarithm. Preliminary analyses found some surprising associations between milk yield and the SOC tests results. In order to characterise the strength of these relationships outside of the regression models, Spearman rank correlations were made between the SOC test results and the average milk yield and days in milk at first test and again between the activity recorded and the average milk yield and days in milk at first test (**Table 6.4**). The explanatory variables included in the maximal model were the home pen behaviour traits recorded in **Table 6.3.** The number of times the cow was successfully milked by the parlour was excluded from the models as it was confounded with the average yield per day. Step count (and standard deviation of step count) was excluded for being too highly correlated with MotionIndex, as was the number of standing bouts which was too similar to the number of lying bouts and total daily lie duration which was confounded with total daily standing duration. The most parsimonious model for

each response variable was then selected using a stepwise method of residual means squares. Explanatory variables were removed from the maximal model when they failed to meet the test criterion of their variance ratio being >4 (Harding & Payne 2011). Some milking traits such as days in milk at testing and average daily milk yield in the 40 days prior to testing are correlated (see Table 6.4), therefore when the final model included both of these variables the sequential and adjusted sums of squares of these models were examined in Minitab to suggest further criteria for eliminating variables (Grafen & Hails 2002). If there is a considerable drop between the sequential and adjusted sums of squares for a particular variable in the model, this suggests that explanatory variables in the model are sharing information. Variables were retained when the adjusted sum squares suggested that they were not explaining variation addressed by other variables in the model. The best models produced by GenStat for predicting the behaviours observed and constructed from the social motivation test are reported.

Table 6.3 *Description of activity and milking behaviour traits in the home pen in the 40 days prior to the behavioural testing period, recorded by the IceQube system and the DeLaval robotic milker.*
**MotionIndex defined in Chapter 5*
†Excluded from predictive models (see text)

Activity Trait	**Description**
Mean Daily Lie and Standard Deviation † (minutes)	The animal's average total lying time in the pen in a day and the standard deviation of this over 40 days. Confounded with Mean Daily Stand.
Mean Lie Bout and Standard Deviation (minutes)	The average duration of a lying bout and the standard deviation of lying bouts over 40 days.
Mean Daily Stand and Standard Deviation (minutes)	The animal's average total standing time (standing periods also include locomotory activity) in the pen in a day and the standard deviation of this over 40 days.
Mean Stand Bout and Standard Deviation (minutes)	The average duration of a standing bout (standing bouts also include locomotory activity) and the standard deviation of standing bouts over 40 days.
Mean Daily Number of Lying Bouts (DNLB) and Standard Deviation † (Count)	Average number of lying bouts observed in a day and the standard deviation of this over 40 days. (Confounded with Mean DNSB.)
Mean Daily Number of Standing Bouts (DNSB) and Standard Deviation (Count)	Average number of standing bouts observed in a day and the standard deviation of this over 40 days.
Mean DailySteps and Standard Deviation † (Count)	Average number of steps recorded (in days where steps were not >4000) and the standard deviation of this over 40 days. Calculated from MotionIndex when tag considered to be in standing position.
Mean DailyMI and Standard Deviation	Average Daily Motion Index* (in days where DMI was not >20,000) and the standard deviation of this over 40 days.
Milking Behaviour	**Description**
Mean Milks/Day†	The average number of successful milking sessions in the robot parlour per day
Mean Refusals/Day	The average number of refused milking sessions in the robot parlour per day
Mean Milk Yield/Day (Kg)	The average kg yield of milk per day from the robot parlour.

6.4 Results

6.4.1 The Social Motivation Test

Spearman rank correlations between the days in milk at first testing, average milk yield and behaviours recorded in the SOC test are presented in **Table 6.4**. Days in milk at first test and the average daily milk yield for the 40 days prior to the start of SOC testing was negatively correlated (r = -0.63, $P < 0.001$).

Table 6.4 *Spearman rank correlations between SOC test results and days in milk at first testing and the average daily milk yield for the 40 days prior to the start of SOC testing. Also shown, spearman rank correlations between activity recorded in the 40 days prior to SOC testing and days in milk at the date of first SOC test and average daily milk yield in the 40 days prior to SOC testing. (* P > 0.05, ** P > 0.01, *** P > 0.001)*

	Days in Milk at the date of first SOC test	Average daily milk yield (Kg) for 40 days prior to start of SOC testing
Behaviours recorded during SOC test		
Duration in 2m box	-0.07	-0.03
Latency to 5m line	>0.01	*0.24
N times in 2m box	-0.20	-0.16
N times in 5m box	-0.06	*-0.27
'Isolation activity'	-0.09	*-0.28
	Activity recorded in 40 days prior to SOC test	
DNLB	-0.07	-0.04
Daily lying bout duration	***0.45	-0.17
Daily MI	-0.01	-0.12
Daily standing bout duration	-0.13	0.10
Daily total standing duration	***-0.56	**0.30
St Dev DNLB	-0.06	-0.13
St Dev Daily lying bout	0.09	0.06
St Dev Daily Standing bout	-0.04	0.08
St Dev total standing duration	-0.10	0.19

The repeatabilities of the observed SOC traits are reported in **Table 6.4**. The latency to both lines was highly repeatable, similar to the repeatabilities for the same behaviours reported by Gibbons et al (2010). However the duration of time spent in the 5m box was not repeatable despite the average between the tests not being

significantly different. This trait was therefore excluded from further analyses, as was latency to 2m line as it was confounded with latency to 5m line. In further analyses, the latency to the 5m line, duration in the 2m box and the number of vocalisations were transformed using a natural logarithm to avoid violating assumptions of normality.

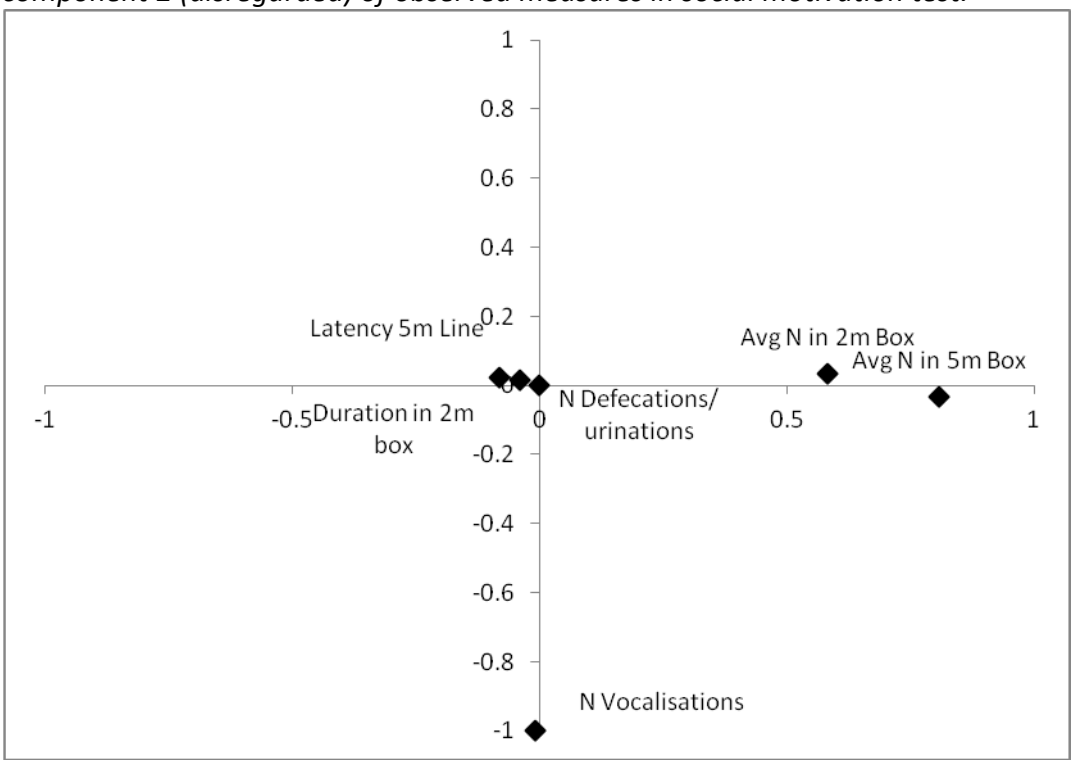
In the principle components analysis, the first component explained 49% of the variation and the second component explained 17% of the variation. While the second component still explained a large amount of variation, this was mostly due to the strong negative loading of the number of vocalisations on this factor and the second component did not describe much else. Additionally, the second component had a much lower eigenvalue (1.01) compared to the first component (2.92) and would not have been selected as a relevant component based on a scree plot (Field 2005). For this reason, the first component was the only component which was included in further analysis; however the second component is included in **Figure 6.2** for illustration. The first component was termed 'isolation activity'. Animals with a high 'isolation activity' score crossed the 5m and 2m lines more frequently when separated from the herd.

Table 6.5 *Means and standard deviations of each measure for groups MS1 and MS2. Spearman's rank correlation between tests and repeatability for all 99 animals shown.*

Measure / Event	MS1 Mean ± S.D.	MS2 Mean ± S.D.	Test 1 Mean ± S.D.	Test 2 Mean ± S.D.	Spearman's Rank Correlation between test 1 and 2	Repeatability
Latency to 5m line (s) ♦	123.30±91.22	130±96.65	122.50±93.07	130.10±94.22	r=0.43, P<0.001	0.49
Latency to 2m line (s) †‡	157.40±106.60	160±105.50	147.70±99.87	170.10±112.10	r=0.44, P<0.001	0.41
Duration in 5m box (s) †‡	49.33±64.29	48.53±54.06	48.41±58.40	49.48±61.57	r=0.121, P=0.241	0.01
Duration in 2m box (s)	172.10±107.40	146.60±102.60	150.50±102.20	170.50±110.00	r=0.35, P<0.001	0.38
N entries in 5m box	1.27±0.79	1.56±1.07	1.61±1.08	1.18±0.69	r=0.33, P=0.106	0.13
N entries in 2m box	0.90±0.51	1.05±0.74	1.08±0.71	0.84±0.50	r=0.57 P<0.001	0.21
N Vocalisations ‡	0.41±1.20	0.51±1.29	0.60±1.36	0.23±1.11	r=0.74, P<0.001	0.38
N Defecations/urinations ‡	0.38±0.54	0.22±0.47	0.32±0.53	0.31±0.51	r=0.52, P=0.027	0.26

† Not included in PCA (see text)
‡ Not predicted by regression models

Figure 6.2 *Factor loadings on principle component 1 (isolation activity) and principle component 2 (disregarded) of observed measures in social motivation test.*



6.4.2 The predictive power of activity and milking yield recorded for 40 days on SOC observed behaviours

See **Table 6.6** for a summary of the significance and direction of associations between activity and milking traits in the 40 days prior to the SOC test and the SOC test results.

Activity and milking traits recorded in the 40 days prior to the start of SOC testing could predict around 6% of the variation in the cow's latency to reach the 5m line. Cows which took a long time to cross the 5m threshold had a larger milk yield in the 40 days prior to the start of the SOC testing ($R^2_{adj} = 0.06$, $F_{1,68} = 5.03$, $t^{(67)} = 2.24$, $P = 0.028$).

The prior 40 days of activity and milk production traits could not predict the duration of time the cows spent in the 2m box, i.e. were in close proximity with the herd. However cows from MS1 and cows who were at earlier days in milk on the day of testing were more likely to spend more time in the 2m box. This model explained 10% of the variation in duration of time spent in the 2m box (R^2_{adj} = 0.10, $F_{2,62}$ = 4.62, P = 0.014; Group (MS2) $t^{(60)}$ = -2.02, P < 0.048; days in milk at first test $t^{(60)}$ = -2.40, P = 0.020).

The model which predicted the number of times the cows crossed the 2m line was more complicated. There were significant associations between the activity and milk production recorded during the 40 days prior to the start of SOC testing and the cow's days in milk at first testing. Cows which crossed the 2m line more often produced less milk per day in the 40 days prior to the test and were also likely to be at an earlier stage of lactation on the day of their test (R^2_{adj} = 0.16, $F_{2,68}$ = 7.27, P = 0.001; days in milk at first test $t^{(66)}$ = -2.72, P < 0.008; average milk yield per day $t^{(66)}$ = -3.77, P = 0<.001). Note that days in milk at first test is included in this model because its adjusted sums of squares suggest it is only significant after milk yield is incorporated into the model. This model explained 16% of the variation in the number of times the cow crossed the 2m line.

In the 40 days prior to SOC testing, cows that had shorter average standing bouts, spent more time standing per day, produced less milk per day and were less variable in the number of lying bouts they had per day, crossed the 5m line more often during their SOC test (R^2_{adj} = 0.19, $F_{4,68}$ = 5.08, P = 0.001; average standing bout duration

$t^{(64)}$ = -3.08, P < 0.003; average daily standing duration t(64) = 2.63, P = 0.011; average milk yield per day t(64) = -3.53, P < 0.001; standard deviation of number of lying bouts per day t(64) = -3.01, P = 0.004). This model explained 19% of the variation in the number of times the cow crossed the 5m line.

6.4.3 The predictive power of home pen activity on Principle Component constructed from SOC behaviours.

Cows which scored highly on the Principle Component of 'isolation activity' produced less milk per day in the 40 days prior to the SOC testing. They were also more likely to be at an earlier days in milk at their first test (R^2_{adj}=0.14, $F_{2,68}$ = 6.60, P = 0.002; days in milk at first test $t^{(66)}$ = -2.23, P < 0.029; average milk yield per day $t^{(66)}$ = -3.63, P < 0.001). This model explained 14% of the variation in the cow's 'isolation activity' score. Again, the performance of the adjusted sum squares indicates that days in milk at first testing was only significant after including average milk yield per day.

Table 6.6 *Direction of effect (+ve or –ve) of predictive variables on responses and percentage of variation explained by the model. (+/- P < 0.05, ++/-- P < 0.01)*

Response and predictive variables	Effect Direction	Total variation explained by model
Latency to reach 5m Line		**6%**
Average milk yield (Kg) per day in prior 40 days to SOC testing	+	
Duration in 2m box		**10%**
Group (MS2)	-	
Days in milk at first test	-	
N Entered 2m Box		**16%**
Days in milks at first test	--	
Average milk yield (Kg) per day in prior 40 days to SOC testing	--	
N Entered 5m box		**19%**
Duration average standing bout in prior 40 days to SOC testing	--	
Duration average daily stand in prior 40 days to SOC testing	+	
Average milk yield (Kg) per day in prior 40 days to SOC testing	--	
St Dev number lying bouts per day in prior 40 days to SOC testing	--	
'Isolation Activity'		**14%**
Days in milk at first test	-	
Average milk yield (Kg) per day in prior 40 days to SOC testing	--	

6.5 Discussion

The gold standard measure of the SOC test, the latency to reach the 5m line (Gibbons et al, 2010) was significantly positively associated with the average milk yield in the 40 days prior to the start of the SOC testing period. Other measures in the SOC test such as activity shown during the test (i.e. number of times the cow crossed the 2m line and the component 'isolation activity') were significantly associated with days in milk at first testing and the average milk yield in the 40 days prior to testing. While the number of times the cow crossed the 5m line was significantly associated with home pen activity behaviours recorded in the 40 days prior to testing, the duration of time the cow spent in proximity with the herd was not. Instead, duration of time spent in the 2m box was significantly associated with the cow's group and her days in milk at the date of her first test. The initial question was whether we could use activity and milking traits recorded in the home pen over 40 days via biotelemetry systems to predict the outcome of the SOC test. These results suggest this is possible, but strongly imply that this is because lactation stage and milk production status were not controlled for in the SOC test as it is currently described. Therefore before we can address whether it is possible to predict a dairy cow's sociability from behaviours recorded in the home pen, we need to discuss whether the result from this study suggests that lactation stage must be controlled for during sociability testing.

As previously stated, the social motivation test appears to satisfy Carter et al's (2012a) criteria for a personality test, as it relates to other measures of sociability, has situational relevance and ecological relevance. The test measures' relationship with the physiological attributes of milk production demonstrated in this study is

concerning. There are several possible explanations for this result. Firstly, the test itself may not measure social motivation in dairy cattle. Alternatively, the test does measure social motivation but was not used properly in this study. Or lastly, there is a relationship between social motivation and milk production. These three explanations will be considered in the following section.

6.5.1 Does the SOC test measure social motivation?

In the introduction 'sociability' was described as the tendency to associate with conspecifics. As previously established by Gibbons et al (2010) the latency to reach the 5m line in the sociability test was significantly associated with how close the cow was to her nearest neighbour in the herd and how synchronous she was with the rest of the herd. We know this test is repeatable for dairy cattle and that it has been used successfully in other species (chicks: Mills & Faure 1990; horses: Lansade et al. 2008) but not, to my knowledge, again in cattle. In cattle, sociability, or gregariousness, is often tested by observing the animal's reaction to separation from the group (Boissy & Le Neindre 1997) or by observing incidences of social interactions in the home pen (Færevik et al. 2008; Wagner et al. 2012) and are often compared with faecal or blood cortisol measures to corroborate that the social challenge is a stressor. That cattle performance is repeatable in such a test is not in question as this study found good repeatabilities in line with have others (Grignard et al. 2000; Müller & Schrader 2005a; Gibbons et al. 2010). Note that the model with the poorest predictive ability is the model which predicts the latency to the 5m line and this is the variable which has been associated most with other measures of sociability and was what Gibbons suggested was the best trait to indicate sociability.

This latency to return, as measured in seconds, could be considered a proxy measure of temperament, much like flight speed is a proxy measure of temperament, rather than a personality trait itself. It may be that other aspects of the test do not reflect sociability to the same extent. For example, the number of times the cow crosses the 5m and 2m lines may be more related to underlying activity (see below) as a 'sociable' cow would remain in proximity with the herd.

Following from this point, the SOC test has the same problem as the other tests utilised in this project in that many of its measures incorporate activity and are often interpreted as the cow exhibiting distress in response to the challenge. The number of times the cow crossed the 5m and 2m lines was not recorded by Gibbons et al but after discussion with those authors, was included in this study. The best model was the one which predicted the number of times the cow entered the 5m box which involved much walking up and down the runway. This model predicted 19% of the variation in the number of times the cow crossed the 5m line. Cows which crossed the line more often during the tests had shorter average standing bouts, spent more time standing per day, produced less milk per day and were less variable in the number of lying bouts they had per day in the 40 days prior to the SOC testing period. The number of times the cow entered the 2m box and the constructed component of 'isolation activity' had similar models to each other which were dependent upon the average milk yield the cow produced in the 40 days prior to testing and her days in milk at the day she was first tested (R^2 16% and 14% respectively). These two line crossing traits (and resultantly, 'isolation activity') may be affected by an underlying activity component which prompts the animal to show

more activity once it has reached its goal of the conspecifics. It is worth noting that the only runway behaviour which showed a relationship with the activity behaviour in the home pen was the number of times the animal crossed the 5m line and if we doubt that the number of times the cow crosses the 5m line is definitely a measure of sociability, there is no evidence in this study to suggest that home pen activity recorded in this manner relate to sociability. Once more, the inherent measures of activity in behavioural tests may be interpreted as pertaining to a personality trait other than an underlying activity trait.

In this study, we utilised more measures from the SOC test than Gibbons et al (2010) did, such as the number of times the cows crossed the line. Our confidence in these measures would greatly benefit from more validation than was afforded to them in this study, such as their relationship with the cow's nearest neighbour distance. However, the latency to the 5m line as an indicator of the cow's underlying sociability is still a reasonable measure as Gibbons et al (2010) had already established this measure, albeit without controlling for lactation status. Previous work looking at social behaviour and home pen activity in cattle has focussed on agonistic indexes (MacKay et al. n.d.; Friend & Polan 1974; Galindo & Broom 2000; Schrader 2002) which is not the same as sociability (Reinhardt & Reinhardt 1981) but still relates to conspecific directed behaviour. Agonistic behaviours have been found to relate to home pen activity (MacKay et al. n.d.; Schrader 2002) so it is surprising that sociability may not. It is possible that the activity measures here were insufficient, but as they related to fear-related behaviours (see Chapter Five) and agonistic behaviours (Chapter Four) that is doubtful. A measure of how synchronous

cows are with other individuals with the herd may be very useful, but again would need to also be compared to nearest neighbour distance for each cow in order to avoid the problems encountered in this study. I would not declare the sociability test ineffective based on the results of this study, however future work using latency to rejoin conspecifics as an indicator of sociability in dairy cows will need to take into account the relationship with milk yield in the prior 40 days found in this study.

6.5.2 Was the study's methodology appropriate?

For logistical reasons, this study was forced to make some departures from the protocol outlined in Gibbons et al 2010, namely a reduced runway size, which reduces the 'effort' the cow must expend to reach conspecifics. The runway in this study was 12m from the start box exit to the herd as opposed to the 18m in Gibbons' study. Additionally, unlike in Gibbons' study, the experimenters were visible at all times, which may have resulted in increased motivation to leave the testing area for cows which were fearful of humans. It should be noted that the observers did not consider the cows to be acting in an unduly fearful manner during the SOC test. Furthermore, cows which entered the 5m box several times were repeatedly moving back towards the visible experimenters and yet this trait also showed associations with milk yield in the 40 days prior to testing. It is possible that the cows are also investigating the observers in this case. Although not obvious to the observers, the cows may be drawn away from the herd to explore or socialise with something not of their species, although the results in this study are not very similar to what we found investigating neophilia in the previous chapter.

On first reflection on the results, I hoped to recalculate the milk yield traits for each cow for a standard number of days of lactation to control for lactation stage. This was not possible for a number of reasons. Data gathered during the experimental period could not be used as cows were restricted from visiting the parlour for certain periods during the experimental setting. Some later work on the farm suggested that my study impacted activity behaviour for a few months following the study's conclusion (Van Reenen, pers. comm.). The most appropriate period for data collection was therefore in the period prior to the testing, which incorporated the 40 days of data collection for the milking traits. This left a limited number of days prior to the study's conclusion with suitable extra data and as all cows were at a different lactation the sample size was greatly reduced. Additionally, this approach would have been further removed from the study's aim of predicting SOC test results from a set period of home pen automated measures of behaviour. There are many other aspects which may need to be controlled for if we believe there is a link between milk production and sociability, such as whether the cow is pregnant, parity, etc. I did remove any cows which had exhibited oestrus behaviours in the days following the test. Future studies should attempt to more closely follow the protocols set out by Gibbons et al and should attempt to control for lactation stage and milk production if choosing to incorporate milking traits in sociability studies.

6.5.3 Is there a relationship between sociability and milk production?

The final possibility is that there is a link between social motivation and behaviours related to milk production in cows milked with a robotic milker. This is not well explored in the literature with most studies of sociability in cattle occurring in calves

(Grignard et al. 2000; Færevik et al. 2006; Færevik et al. 2008) or young heifers (Boissy & Le Neindre 1997; Raussi et al. 2005). We do know a little of how an automated milking system influences milk production. Automated milking systems tend to result in more evenly distributed activity patterns compared to traditional parlour systems and increased waiting in the holding area outside of the robot milker at times of human intervention (Wagner-Storch & Palmer 2003). Higher milk yields and milking frequencies have also been seen in automated systems (Winter & Hillerton 1995; Wagner-Storch & Palmer 2003). Early lactation stage cows who are milked more frequently have a higher milk yield in later stages of lactation and a low milking frequency forced on the cow at an early lactation stage cannot be compensated for after peak lactation (Svennersten-Sjaunja & Pettersson 2008). The Svennersten-Sjaunja & Pettersson review also concluded that automated systems promoted the continual mixing of cows as new cows can be introduced to the herd regularly. As previously mentioned, mixing is a social stress on cattle and is one of the primary reasons we are interested in the underlying sociability trait. If sociable cows are reacting to the stress of continual mixing by visiting the parlour less often at an early stage of lactation, their milk yield may be negatively affected, but this is only speculation. It may also be the case that the more social cows in this herd are more stressed by continual mixing and this stress is resulting in inhibited milk production, as stressful events can inhibit the release of oxytocin disrupting milk release (Bruckmaier & Blum 1998; Van Reenen et al. 2002). Previous studies attempting to link aspects of personality and emotions to milking behaviour have often concentrated on the cow's relationships with humans (Purcell et al. 1988), whereas studies looking at positive human interactions have found a link with milk

yield (Bertenshaw et al. 2008). It may be that cows are simply more sociable at earlier, higher yielding stages of lactation. Gibbons found that sociable cows were also more present at the feedface at peak feeding and speculated that feedface presence may be another indicator of a sociable animal, so the relationship between feed intake, sociability and milk production should also be studied in more detail. If this is the case, we would have to reconsider how we define sociability as a personality trait, as it may not be as consistent across time as we first thought.

More research is needed into how more sociable and less sociable cows respond to the stress of modern management practices. A reliable method of assessing sociability is needed before we can state whether sociability has an effect on milk production. If such a link exists, careful thought must be given to how the dairy industry should tackle the issue.

6.6 Conclusions

In this study I aimed to use activity and milk production traits gathered via automated biotelemetry systems to predict a cow's performance in a SOC test. Unfortunately, due to the surprising relationship between milk production and the cow's sociability levels, this question cannot be further addressed until the link between sociability and lactation stage is further investigated. It would be particularly valuable to compare automated milking systems and traditional parlour systems, as well as to manipulate social stress to obtain a better idea of the size of this effect. It is very important in any further studies of sociability that the lactation

status of the cows is controlled for. This must be investigated before we can attempt to remotely assess sociability in dairy cows.

Chapter Seven

General Discussion

7.1 Introduction

Individual cattle possess personalities. Their behavioural response to an environmental challenge can be consistent through time and across situations and will consistently differ from others in their population (Cafe et al. 2011; van Reenen 2012; Stockman et al. 2012). These underlying personality traits affect how the animal interacts with and perceives its environment (Turner et al. 2011a). As well as the impact on welfare (Curley et al. 2006), personality can also affect production. If the personality of the animal makes it more prone to stress, meat and milk production can be negatively affected (Voisinet et al. 1997a; Voisinet et al. 1997b; Hemsworth et al. 2000; Cooke et al. 2012). By testing cattle using standardised challenges designed to provoke a certain response we can gain information on how they react emotionally to their environment. Despite the usefulness of such tests, we are only observing the animal in artificial and stressful conditions and then relating these behaviours to other traits (Biro 2012).

Previously, unobtrusive assessment of personality traits in the home environment was done by concealed observers using a rating method (Highfill et al. 2010) where an experienced observer makes a qualitative assessment of the animal's behaviour. Without careful experimental design the observer may affect the behaviour of the animal in question (Martin & Bateson 1993). With recent advances in biotelemetry systems, activity monitors capable of recording for long periods of time are now available for cattle and can be fully integrated into the farming system, providing information for the farmer such as on occurrence of oestrus and health monitoring

(Firk et al. 2002; Rushen 2012; Rutten et al. 2013). Do these systems record behaviours related to personality traits and if so can we use them to remotely evaluate cattle welfare? By utilising one such system, the IceTag activity monitor, this project investigated whether personality traits persist outside of the test environment and go on to affect the animal's day-to-day life.

The aims of this project were:

1. To gain an understanding of the terms 'personality' and 'temperament' when used in the context of animal behaviour to ensure the terminology is used consistently within the project.

2. To evaluate whether fitting IceTags to cattle could affect their behaviour and so would limit the application of tags in future behavioural studies.

3. To investigate how variation in behaviours displayed in short duration tests of temperament and personality relate to activity behaviours recorded in the home pen using IceTags over a longer duration of time.

Work was carried out across three experimental farms in the UK and the Netherlands in order to investigate the limitations and capabilities of the IceTag device in this context and how short term personality tests relate to home pen activity in cattle.

The purpose of this chapter is to discuss the results of Chapters Three and Four regarding the mechanics of how tags are used and whether previous methodologies of establishing activity bouts with IceTags were robust. This chapter will then discuss the main findings of Four, Five and Six in context of behaviour and welfare,

particularly with note to how remote sensors have enabled us to address hitherto unknown aspects of cattle behaviour with respects to personality.

7.2 Evaluating the use of the IceTag biotelemetry system

7.2.1 Effects of IceTags on cattle behaviour

One of the fundamental aspects of this body of work was the idea of recording spontaneous cattle activity within the home pen. This could not be done without first establishing whether IceTags influence the behaviour of the cows they are attached to. A tag which discouraged a cow from lying would not record data which could then be generalised to a non-tagged population, limiting the application of this device in behavioural studies. Chapter Three reported the results of a short study observing the lying and standing activities of cattle with and without activity monitors. Lame cows and cows which were naïve to the tag fitting process were incorporated in the study. Although a small effect was observed within the first two days, overall there was no statistically significant effect of being tagged on dairy cow behaviour.

Previous to this study, the possibility of affecting cattle behaviours through the fitting of a biotelemetry device had not been raised. Despite this, we knew that cattle behaviours would be affected by stimuli such as the colour of a transceiver in the environment (Swain 2003) and cattle would show considerable kicks of a tagged leg immediately after tagging (personal observation). Studies using IceTags often use a habituation period, although the duration of this period has been variable with previous studies using data from accelerometers within a few hours (Blackie et al. 2006; Aharoni et al. 2009), 24 hours after tagging (Bewley et al. 2010) or fourteen

hours after tagging (Gibbons et al. 2012). The possibility of an effect has since also been investigated by Gibbons et al. (2012) who came to a similar conclusion as found in Chapter Three, that there was no effect of a tag on lying times and lying laterality. Both studies were only designed to establish whether an effect existed. Had we found evidence of an effect we would have required more animals and studies to understand the extent of the effect and how it could be combated. The small sample size of this study is also complicated by the split plot factorial design and the inclusion of lame and naïve animals. With the small sample sizes of both these groups we cannot truly be confident that the effect on the lame animals was not significant. Lame animals were not used in the rest of the project and I would continue to recommend that future studies incorporating lame animals are aware of possible effects. They should always record extra information such as lameness incidence in case of an effect detected in lame animals after the study's conclusion. In the case of lame animals, I believe that further data on the pressure and torsion of a foot with a tag attached to the leg would also be beneficial to understand how the tag changes the way an animal walks. Chapter Three was initially designed with a second biotelemetry system, the BouMatic pressure plate which automates the recording of lameness, intended to supply extra data on weight distribution immediately post-tagging. Difficulty in retrieving data from this device and extremely variable recordings from the device within individual cows in short, non-tagged time periods meant that it was not possible to include the pressure plate data in the analysis. If one wished to conduct further research on the impact of tags on lame animals, pressure plates would be useful, but those that are commercially available require more evaluation than could be afforded in this project.

The conclusions of Chapter Three were extended into the experimental design of Chapter Four, resulting in a two day habituation period after tagging for the steers. This does mean that the results were generalised from dairy cows to beef steers. There are some differences between breeds, in maternal behaviours and overall descriptions of temperament (Grignard et al. 2001; Hoppe et al. 2008) and beef cows are generally selected for muscle quality versus milk production in dairy cows. Again, had there been more evidence of a strong effect of tags on the lying behaviour of dairy cattle, it may have been necessary to run a short pilot study on beef cattle. However it seems more likely that the size and placing of the device, when fitted correctly, does not affect cattle, as they are large, robust animals and I am confident in using the devices and describing them as 'unobtrusive' after a forty eight hour habituation period.

7.2.2 Considerations of home pen activity measures

In Chapter Four, four groups of beef steers were tagged for two 14 day periods, with 14 days between each tagged period. This experimental design with two periods of tagging afforded the opportunity of calculating the repeatability of activity traits. The repeatability of lying and standing periods of activity in dairy cattle was previously addressed by Schrader (2002) and Müller & Schrader (2005), but not to my knowledge in beef cattle. The purpose of reassessing the repeatability of activity data was mainly to investigate the repeatability bout criteria established by Tolkamp et al. (2010), but it also established the repeatability of home pen activity in beef steers. Utilising steers also allowed for the exploration of activity without factoring in

oestrus cycles and milking routines and so this study was judged to be an excellent opportunity to construct different variables from the IceTag dataset. By expressing activity as a function of time (i.e. MotionIndex per minute standing) the possibility of finding variables expressing home pen activity other than average lying and standing durations was also explored. The high repeatability of the average lying bout (0.67) and standing bout (0.70) durations strongly encouraged their usage over other measures such as the mean minimum bout durations which had lower repeatabilities. This result also supported the bout criteria proposed by Tolkamp et al (2010). Overall, when paired with Müller and Schrader's work on dairy cows, home pen activity traits appear to be highly repeatable. In Müller & Schrader (2005), activity in dairy cattle was recorded as the number of high and low activity bouts and the duration of low activity bouts using a different automated method of recording activity from the IceTags. Repeatability was calculated using the same covariance method used in Chapter Four but over much longer periods with 6 weeks between intervals 1 and 2, 2 and 3, and 30 weeks between intervals 3 and 4. The repeatability estimate over the four sample points estimates ranged from 0.62 for the number of high activity bouts, 0.40 for the number of low activity bouts and 0.49 for the duration of low activity bouts. Schrader (2002) did not use the covariance method of calculating repeatability but correlated the duration of lying periods and time spent standing across repetitions and found an r_s from 0.44 to 0.73 for lying period duration and 0.45 to 0.67 for standing duration. These results suggest that home pen activity, as recorded by activity monitors which do not allow for ambiguity, i.e. the tagged animal is either standing or lying, is highly repeatable for a behavioural trait. In their review of the repeatability of behaviour, Bell et al. (2009) advocated the

covariance method as used by Lessels & Boag (1987) as was used in this project. They also found that the average repeatability estimate of behaviour was 0.37, repeatability estimates of home pen activity in cattle can be considered high. It is important to note though that the repeatability estimate is incorporating the tag's assumptions of standing and lying and it is more true to say that the tag's records of activity in the home pen are repeatable across time

7.2.2.1 The use of MotionIndex

Chapter Four was also one of the first studies to explore MotionIndex, an activity parameter which is still somewhat neglected by researchers utilising IceTag technology and is rarely mentioned in publications. In my experience, many researchers will prefer to use step-count and this is reflected in the step-count's presence in the literature (Ouweltjes et al. 2009; Aharoni et al. 2009). MotionIndex is a truer measure of acceleration (g) as it reflects acceleration recorded on all three axes. IceRobotics defines MotionIndex in their product catalogue thus:

> *"MotionIndex indicates the overall activity of the cow calculated using the acceleration on each of the 3 axes. This is a proprietary measure and is recommended over the Step Count as a measure of activity."*
> (IceRobotics 2010)

Some of the concerns raised about MotionIndex by the research community are that it is not expressed in units, that IceRobotics will not allow their proprietary algorithm to be published and that it is not truly an index despite being trademarked under that name. These views have been frequently discussed at conferences attended throughout the project. These concerns typify the difficulties of using a commercial

'off the shelf' biotelemetry system. By purchasing the validation and software the company has invested in, the researcher also purchases the assumptions made by the software. In Chapter Four, both MotionIndex and step-count had similar relationships to the temperament test measures used. This is because they are highly related. Summed acceleration from more open source devices has been found to be informative of cattle behaviour (Rushen & De Passillé 2012). Proper acceleration is the measurable acceleration relative to freefall (Taylor & Wheeler 1966) and MotionIndex therefore is simply a calculation of this value across the three axes for a given time period. Although we do not see 'inside the black box', we do know that MotionIndex, when the tag is presumed to be in a standing position, is converted to step count. Therefore any acceptance of step count implicitly accepts MotionIndex. In rare instances when step count is not applicable (i.e. due to the tag not being fixed to a leg or on a species of animal where step-count has not been validated) the MotionIndex still provides valuable information about the amount of movement the tag records. I strongly advocate the usage of MotionIndex in any IceTag study.

7.2.3 Future directions in the use of activity monitors

In this thesis, activity was recorded over distinct periods and an average daily total was used as a variable. This was because one of the main advantages of using routinely recorded herd data, such as data from a milking parlour or an activity database for oestrus detection, for welfare assessment is the reduction in time needed to perform the assessment (De Vries et al. 2011). In this thesis, spontaneous activity in cattle recorded by activity monitors has been shown to relate to behavioural testing measures. While the total variation in activity explained by personality is sometimes

small (see section 7.3) this conclusion raises two questions specifically about the future usage of activity monitors. Can these systems be applied at a farm level to assess welfare and how can activity be assessed in a continuously recording setting? As mentioned by Rushen (2012) databases which continuously record activity for oestrus detection and other measures on farm are becoming ever more popular in agricultural management systems. It is this kind of database which is most likely to be targeted by welfare assessors in the future. With long periods of data available for use, selecting an appropriate sampling interval is very important.

7.2.3.1 Choosing the appropriate sampling interval

One possible method of tackling the issue of what is the most appropriate sampling interval is to use signal processing techniques to analyse activity records. Signal processing comes from the field of electronic engineering and is a method of cleaning an analogue signal in order to more accurately present it. Its usage in biological systems is related to signals, such as ECG monitoring and other biotelemetry devices (Frost et al. 1997). Activity data is an ideal candidate for these techniques and individual data records have been treated with signal processing methods before to inform on the individual's patterns and cyclicity of behaviours (Scheibe et al. 1999; Berger et al. 2002; Berger et al. 2003). These methods treat an activity pattern as an analogue signal and convert it to a waveform. Berger et al. (2002, 2003) use autocorrelations as an intermediate step in their investigation of patterns in individual animals. Autocorrelations find an underlying periodic signal, or waveform, in a set of data with *n* number of time points between them. The autocorrelation function works by looking at the data point at time point #1 and

correlates that to the data point at #2. There is one time period between #1 and #2 so this has a lag of 1. The autocorrelation then correlates the data at #2 and #3, #3 and #4 and so on to give a correlation for all points with a lag of 1 between them. A lag of 2 would tell us how correlated points #1 and #3 are, and points #2 and #4 etc. If our time point was hours and there was a strong daily influence in our subject animal, i.e. the cow always stood to eat at 8 A.M. when feeding occurred, there would be a strong correlation, or waveform peak, at a lag of 24 hours because every day at 8 A.M. If the cow was very regular then the waveform would be more cohesive, however a cow which was highly variable in its activity patterns would have a less cohesive waveform. In essence, a cleaner waveform would be a more predictable and cyclical activity pattern. As disruptions to activity (Tolkamp 2002; González et al. 2008) can be indicators of disease onset, this is an attractive method.

This kind of methodology has previously only been used on single animals, not groups or herds, and this is a possible avenue for future research. There is some issue with how it could be used on an individual within a herd. The behaviour of one individual herd animal is always affected by the others in the herd and this has often raised the issue of what a replicate is in studies concerning herds (Phillips 1998; Rook 1999; Phillips 2000). In this project there were only two samples of herd activity patterns, MS1 and MS2 and both were from the same farm and so it is difficult to draw any meaningful conclusion based on signal processing techniques applied to the activity patterns of the groups used in this thesis. Future studies need more herds with long term activity data over a number of seasons in order to make comparisons across farms, in both fully housed and outdoor systems and in all-year-

round and seasonal breeding systems. The use of signal processing methods on herds also requires validation with regard to animal welfare indicators, but is an important avenue for future research, particularly on farms where activity monitoring systems are already in place.

7.2.3.2 Possible improvements to sensors

This project was a BBSRC CASE partnership with IceRobotics and so focussed mainly on IceTags as an activity monitor, with some usage of HOKO bin feeders and DeLaval robotic milking parlours. In section 7.2.2.1 some of the difficulties of using commercial systems were addressed. This section will discuss the software assumptions and software limitations and how these may be improved in future.

The ideal sensor device would be one which reported data with no inherent ambiguity. This requires the sensor to log a high quality, always accurate signal, i.e. the device never records a false or erroneous reading, and for the interpretation of that signal to always be correct, i.e. the signal that is recorded always clearly corresponds to a certain behaviour (Ropert-Coudert & Wilson 2004). IceTags incorporate ambiguity in several ways. In Chapters Five and Six several records were eliminated from the CattleGrid database for recording abnormal values (see section 5.3.4 for values). These records were considered to be ambiguous because from previous work in the project and guidelines established by Tolkamp et al. (2010) it was judged to be unlikely that these records could reflect true activity patterns. The assumption is that this was a device failure or, more likely, an interrupted download which generated these abnormal values. These steps in the methodology imply an

inherent lack of trust in the activity recorded by the device. It is fair to say that this project did not work with true activity but activity assumed by the IceTags.

The problems of device failure (and associated device failure, such as transceivers) are going to be inherent in any system recording continuous data. Similarly, fit failures, such as unexpected device loss or movement so the device is no longer positioned where algorithms assume it to be can result in erroneous readings. Some device failures, such as a failure to report any data, can be easily flagged up by the database and the device can be repaired or replaced. Other failures, such as a slight miscalibration of a sensor, can be all but impossible to detect from the database. With time, new developments in sensor technology will improve their robustness and error reporting (Cooke et al. 2004) and these will improve the ability to assess behaviour and welfare remotely.

7.3 The persistence of personality across contexts

In many ways, Chapter Four was a 'proof of concept' study. As well as exploring what types of information can be retrieved from the IceTags, it also established that a relationship exists between behaviours recorded in testing environments outside of the home pen and activity recorded by the IceTags in the home pen. It was this result which allowed further exploration of the possibility of using biotelemetry devices to characterise individual variation in cattle behaviour.

Chapter Two framed personality research around a point of reference, be that the population or many populations, in one context or in general contexts. In Chapter

Two, temperament was considered to be important because it could be quantified with proxy measurements, such as flight speed. Chapter Four established that flight speed as a proxy measure of fear in a handling context and the ability to displace other steers at a feeding station both significantly correlated to activity recorded over many days within the home pen. Although these correlations were significant, they were not very strong, and there may be several reasons for this. Firstly, as discussed in the chapter, flight speed and displacement index are proxy measures, i.e. a $1ms^{-1}$ increase in flight speed does not imply a 1 unit increase of fear. Furthermore, fear itself is not easily quantified, if at all. In Chapter Two, fearfulness as a personality trait was defined as the predisposition to act in a fearful manner towards a stimulus compared to the population and to consider it as a scalar variable may not be exactly correct (Uher 2011). Hence, the weakness in the correlation may come from the difficulty in using proxy measures. Alternatively, as discussed above, the sensors themselves may not be accurate or sensitive enough to detect activities related to fear. If the sensors also incorporated heart rate, internal temperature, blood flow, etc., they may have found a stronger correlation with these other measures. Activity alone, as measured by the IceTags, may not be greatly affected by personality. Regardless, these correlations were significant and so the discussion of Chapter Four concluded that temperament testing reflected a general trait which goes on to affect the lives and welfare of cattle in their day-to-day lives. While there is a long history of establishing the repeatability of temperament traits in cattle over time (Burrow 1997; Turner et al. 2011a; Haskell et al. 2012) this has mainly been between the same tests over time. A strict interpretation of the repeatability of temperament traits would be that in test situations, cattle behave consistently. It may not immediately

follow that the trait would continue to underpin behaviours exhibited in the animal's day-to-day life, although this is a fundamental aspect of how we believe personality affects welfare. Two main types of behaviour were investigated in this project: fearful behaviours and conspecific directed behaviours, i.e. aggression and sociability. By investigating constructed components of behavioural variation which reference the population (e.g. via principle components analysis) personality can be addressed more directly.

7.3.1 Fear in cattle

In both populations, the steers and the dairy cows, fearfulness exhibited in short tests related to differences in home pen activity patterns seen over a number of weeks. Steers which escaped faster from a handling crush were more active and took more steps within the home pen and consumed less food. Neophobic dairy cows had more lying bouts in a day and a more variable lying bout duration. Likewise, cows which could be characterised as bold had a less variable lying bout duration. The flight speed test and the NANO test are very different, with one reflecting escape from a stressful situation and the other explorative behaviours during a stressful situation, which may explain why they reflect different aspects of home pen activity (Carter et al. 2012b). There are also fundamental physiological differences between the steers and the cows. The steers were a much younger population overall (474 days ± 16 days) compared to the dairy cows (1493 days ± 530 days at first test for MS1). The steers were removed from their pen once a week for weighing, but aside from that were left to mature in their pens with very little interference. Over the period of activity data collection for the dairy cows, they milked an average of 3 ± 0.64 times

per day and were more familiar with human presence in the pen for various management reasons such as health checking, etc. Their home pen environments were very different also with the dairy cows housed in a typical cubicle and passageway system and the steers in straw bedded pens. There may have been less opportunity for the dairy cows to exhibit higher activity levels as dairy cows only have free movement along passageways which are long, rectangular areas, whereas beef steers had free movement over their whole pen which was a large, square area. Additionally, dairy cows often stand and occupy passageways so a cow who wishes to pass must push past occupying cows, which may be a competitive event and further limits the dairy cow's free movement. It would be interesting to compare this result with similar work carried out in dairy cows with access to outdoor pasture or a loafing area. If the activity of fearful dairy cattle is increased in these areas, this may be an argument to include loafing areas for the expression of natural behaviours.

The greater standard deviation of lying bout duration seen in cows which were neophobic is particularly interesting. One hypothesis to explain this behaviour is that cows which are more prone to fearful reactions will cut short a lying bout if a fear eliciting stimulus is present, moving to resume the lying bout elsewhere or at another time point. If so, this disruption of important behaviours could illustrate the impact fear can have on the animal's day-to-day life but also on how it can affect production. To test this hypothesis, one could employ a fear eliciting stimuli in the home pen at known time points to identify whether neophobic cows truly cease their lying bouts at this time, leading to more variable lying bout durations. By introducing known fear-causing events, the strength of these relationships may also be improved.

The experimental work in this project aimed to find relationships between behaviours recorded in testing environments and spontaneous home pen behaviours, but in a well managed farm there may not always be stimuli present which will elicit fearful reactions, indeed, these events are minimised on well managed farms. This is one more possible reason for the low R^2 values seen in this study. By manipulating the home environment and introducing more fearful stimuli, we may see more altered activity patterns in fearful cattle. This approach would not necessarily be useful for reducing the workload on-farm assessment of welfare but it would assist in understanding how fear affects activity patterns.

There is a wealth of research investigating the negative effects of fear on welfare and production (Boissy & Bouissou 1995; Hemsworth 2003; Welp 2004; Van Reenen et al. 2005; Turner et al. 2011b) but the mechanism for this continued impact has been only supposition (Biro 2012). It is understood that an animal's fear can influence not only its behaviour but the stockperson attitudes to the animal (Raussi 2003). By demonstrating that fear has a significant impact on activity budgets, building upon this body of work may begin to provide evidence for why fear can have an impact on production traits.

The greater relevance of this work, however, is that a predisposition for feeling fear is a trait that the animal must live with every day. Activity data gathered over a period of many weeks can be related to cattle's response to three different tests of fear, a flight speed test, a novel arena/novel object test and a human approach test. While these three tests present three different fear eliciting stimuli (Carter et al.

2012b), each one is relevant to home pen behaviours in some way and this helps to address some of (Forkman et al. 2007) criticisms of the lack of validation in tests of fear used in farm animals. It may be too easy to consider this as an exercise in demonstrating the persistence of behaviours across contexts without recognising the emotional component involved. Darwin (1872) discussed the expression of emotions in animals at great length and describes 'terror' thusly:

> *"With all or almost all animals, even with birds, Terror causes the body to tremble. The skin becomes pale, sweat breaks out, and the hair bristles. The secretions of the alimentary canal and of the kidneys are increased, and they involuntarily voided, owing to the relaxation of the sphincter muscles, as is known to be the case with man, and as I have seen with cattle, dogs, cats and monkeys. The breathing is hurried. The heart beats quickly, wildly and violently; but whether it pumps the blood more efficiently through the body may be doubted for the surface seems bloodless and the strength of the muscles soon fails."*(Darwin 1872)

We typically regard fear to be an unpleasant emotion, and Darwin's somewhat stirring account of fear is an example as to why we do so. Since Darwin's study of the emotions in animals, there has been a resistance to the idea of animal sentience and a change in perception regarding whether animals feel emotion. This was lamented by Dawkins (2006b). Dawkins goes on to discuss how animals' perceptions of their environment can cause them emotional suffering. By exploring the possibility of identifying fearful individuals remotely, this thesis takes another step in improving the welfare of cattle in different management systems.

7.3.2 Social behaviours in cattle

Agonistic and dominance related behaviours in steers and social motivation in dairy cattle were also investigated. Steers capable of displacing other steers at a feedface lay for shorter time periods overall, had longer standing bouts, higher food consumption and a more variable daily standing duration. Dairy cows which showed a greater latency to rejoin their conspecifics (i.e. were less socially motivated according to typical interpretations of the test) produced more milk, although the results of this study also suggest that lactation stage must be controlled for in studies of sociableness. The applicability of the social motivation test and the limitations of that study were discussed in great detail in Chapter Six. At this point it is interesting to note how what may be viewed as a positive trait, the desire to seek out social contact, may in fact have negative impacts in a production system. In group living animals, the types of interactions an individual has with others in the group are a significant component of that individual's environment. Social behaviours fulfil many roles such as comfort (Laister et al. 2011), the transmission of information between individuals (Nicol 1995) and promoting exploratory or feeding behaviours (Patison et al. 2010). The experience and personality of an individual animal in a certain role can affect the group's behaviour. For instance, more mature and successful matriarchs in elephant herds promote closer physical grouping in response to vocal cues (Whitehead 2008). While there has been research on how regrouping and being isolated from other cows affects the dairy cow, little work has been done on how less sociable dairy cows adapt to these challenges.

If, after further research, the negative relationship between milk production and sociability remains constant in dairy cattle, there may be a desire to breed for cows

which will not prioritise social interactions. Unsociable animals already exist within the herd so why should there be any concern in selecting for them? Are non-sociable animals more likely to become aggressive, or less capable of interpreting social cues from their environment? The decision to compete is often made based on prior experience and interactions (Chiyo et al. 2011), if an unsociable cow avoids social interactions, will she lose the ability to compete at the feedface? Perhaps more worryingly, has selection for unsociable cows already occurred without our knowledge? In breeding for robust cows, there was some evidence to suggest that highly robust heifers were less sociable in terms of social synchrony and fed at non-peak times (Lawrence et al. 2009). Is this the choice of the animal? It is possible that the animal finds no benefit in social interactions and prefers to feed when there are fewer cows at the feedface, in which case being unsociable does not necessarily imply that the animal is missing out on social interactions which are positive to welfare. Alternatively, the animal finds social interactions to be unpleasant or is somehow incapable of participating in a social interaction successfully. In dogs, selective breeding for certain physical characteristics in smaller breeds had left them incapable of submissive behavioural signals (Goodwin et al. 1997). If these unsociable dairy cows are in fact unsuccessful at social interactions through some fluke of selective breeding, this may well be another welfare issue which needs addressing. Therefore, as well as controlling for lactation status, future studies may also want to control for animals with a high genetic yield to investigate whether they are less sociable. There is clearly a great deal of work still to be done to understand how sociableness affects the individual cow's life.

Social behaviours are different from fear in some very fundamental ways. Fear is an immediate response to a specific situation, provoking an appropriate response to danger. As previously discussed, social behaviours perform a range of functions. While we are keen to remove fear eliciting events from management systems, we may not necessarily wish to remove social events from the management system as social bonds can be advantageous in cattle (Von Keyserlingk et al. 2008; Val-Laillet et al. 2009). Rather it may be more important to address the causes of social stress to see if this improves milk production first.

It is unfortunate that the results of Chapter Six did not allow for a more solid conclusion regarding social motivation and dairy cow behaviour. Regardless, in steers the ability to displace other steers from the feedface was significantly correlated with home pen activity behaviours. Once more, there is evidence to suggest that behaviours in testing situations are linked to spontaneous behaviours in the home pen.

7.4 The possibility of a behavioural syndrome in cattle

Throughout this thesis, the possibility that activity itself is a personality trait has been raised. If so, it is a trait that is difficult to tease out because our methods of assessing personality tend to be dependent on activity shown in a testing environment. This may be an example of a behavioural syndrome where fear and activity are related across contexts in the cattle population. Behavioural syndromes are often explained as a constraint hypothesis (Sih et al. 2004), that is to say they limit behavioural plasticity to ensure an individual does not spend too much time in a ‘wrong’ or

maladaptive behavioural phenotype. Carter et al. (2012a) warned against the use of so-called 'standard' personality tests, exactly the type used in this thesis, because it may lead to misleading or false labelling of personality traits. It is possible that the existence of a behavioural syndrome which creates a correlation between activity and fear has resulted in the mislabelling of the fearfulness personality trait in cattle and that is what will be considered in this section.

In humans, physical activity has been shown to correlate with the personality trait of extraversion (Kuh & Cooper 1992) and extraversion has been considered to be similar to the trait of boldness in animals (Gosling & John 1999). Many human studies have looked at the possibility of personality being a barrier to regular physical exercise, with one review finding a negative effect of neuroticism on exercise and a positive effect of extraversion and conscientiousness (Rhodes & Smith 2006). Voluntary exercise, with all the health benefits and social constructs around it, is not the same as a basic, underlying predisposition to be active. Fidgeting, the expenditure of energy in a physical manner whilst otherwise at rest, was considered a personality trait by Plomin & Foch (1980) in a study of identical and fraternal twins. The variance in fidgeting was mostly accounted for 'between-family' factors (i.e. factors which make family members similar to one another and not similar to someone from another family, as opposed to 'within family' factors which make members of a family different from one another). Likewise, 'between-family' factors accounted for most of the variance in activity recorded by a pedometer over the period of a week. Plomin & Foch called for more genetic studies of personality traits in twins as a consequence, but less work has been done on fidgeting. Some studies of

fidgeting in humans have associated it with anxiousness (Mehrabian & Friedman 1986). Is it possible that within cattle there tends to be a correlation between fearfulness and activity? Is this part of a greater behavioural syndrome? If so, we cannot necessarily infer information about fearfulness from activity. This accidental labelling of one trait as two is also referred to as a 'jangle' fallacy (Uher 2011).

A review by Carter et al.'s (2012a) of the use of personality tests in animals came up with three methods to ensure that personality tests were appropriately identified. These were (1) Consideration of test design, (2) Develop multiple tests for the trait of interest and (3) Validate the tests used. With regards to the first and third points, the specific tests used in this thesis have all been methodologically validated (Van Reenen et al. 2004; Kadel et al. 2006; Gibbons et al. 2009a; Gibbons et al. 2010; Turner et al. 2011a; Haskell et al. 2012; Vetters et al. 2013). The social motivation test is a possible exception, although well established by Gibbons, has still to be widely adopted for usage in cattle. It is well established in hens (Mills & Faure 1990; François et al. 1998; Ghareeb et al. 2008) and if it is more widely adopted within cattle, the test's validity in cattle will be further assessed. The tests are also not dependent on behavioural coding or qualitative measures which are influenced by observer experience (Highfill et al. 2010) but some of the quantitative measures in the tests have been found relate to those qualitative measures (Vetters et al. 2013). The tests all have situational relevance. Flight speed tests take place in an environmental challenge all steers will encounter (handling), the novelty test presents novel stimuli and environments which may occur in the cow's life and the human approach test is relevant to any situation with close human contact. I have already

discussed how, in the example of fear, the tests all examine different aspects of fear. While multiple tests were not used for all traits of interest, I did examine social-related behaviours and fear in different contexts, as well as looking at home pen behaviours as well. I am confident, therefore, that the best tests available for cattle were used in this experiment, although with more time I would have liked to have further validated the social motivation work with nearest-neighbour distances. The question is not whether there were better tests, but rather, is our interpretation of the tests results too reliant upon activity as a measure of distress?

The possibility of a behavioural syndrome linking activity and fearfulness in cattle is potentially very disturbing for researchers. For one, the emotional component of fear is not necessarily present in activity and so the use of these tests in welfare assessment comes under criticism. As stated above, the tests used are well established and satisfy the criteria for measuring a personality trait. However if higher levels of activity tends to present along with higher levels of fear, when are we not picking up fear because of a lack of activity in a fear eliciting environment? This is another way of approaching the idea of 'passive copers' (Koolhaas et al. 1999; van Reenen 2012). Indeed the hypothesis of a behavioural syndrome existing between fearfulness and activity and active/passive coping are very compatible. Passive copers would deviate from the syndrome, showing little activity with fearfulness. There is some evidence of this in chicks where heart rate does not always correlate with active behaviours in the way we might expect with so called 'docile' hens showing few escape behaviours but continue to have elevated heart rates after the fearful stimuli were removed (Duncan 1979). Chapter Five presents some

evidence to contradict this idea of passive copers not being taken into account. The difference in the amount of variation in the constructed trait of 'neophobia' and the observed traits it was comprised of that could be explained by home pen activity suggests that the NANO test is measuring something other than a general underlying activity component. Furthermore, the component 'vocalisations', if picking up a non-active measure of stress, did not have a significant relationship with the fear-related activity components. It is still possible that passive copers exist and to investigate this more fully, a different approach is needed.

7.4.1 Identifying fearfulness without activity

Chapter Two lamented the inability to quantify personality traits such as fearfulness as there was no unit with which to mention fear. Affective states are generally assumed to occur within the neurological system, as evidenced by the rise of new disciplines such as affective neuroscience (Boissy et al. 2007). Is there evidence to suggest personality variation between individuals has a neurological basis, and if so, can we identify fear when it is being felt, if not expressed behaviourally?

Within humans there is evidence for a neurological basis of personality. Changes in the 'big five' personality traits in humans can predict Alzheimer's disease with a general increase in neuroticism and decrease in extraversion present in Alzheimer's sufferers (Chatterjee et al. 1992; Balsis et al. 2005). However starting with a high neuroticism score or low extraversion score is not a significant predictor of later Alzheimer's development (Wang et al. 2009), it is the personality *change* that is the predictor (Balsis et al. 2005). This suggests that the neuropathology of Alzheimer's

is the cause of the change. Alzheimer's affects the brain, resulting in shrinkage of certain structures and larger ventricle spaces. This is not to imply that neurotic personalities are associated with larger ventricle spaces but that physical changes to the brain can change personality. Another early symptom is the impairment of memory recall. As personality is affected by experience (Rothbart 2007), impaired recall may affect this component of personality.

There is also evidence of emotional states directly affecting neurology. One set of studies investigated neurological development in relation to fear experienced by rats (Wellman 2001; Cook & Wellman 2004; Brown et al. 2005; Miracle et al. 2006). In an experimental setting, rats were conditioned to fear an audio tone through its association with an electric shock. The rats were then taught not to fear the tone through extinction trials where the tone was played without an electric shock. When the animal no longer displayed fearful behaviours upon hearing the tone, its fear was considered extinct. When the rats were stressed, the researchers found that their recollection of the extinction training was impaired which they theorised was due to dendritic shortening in the medial prefrontal cortex as a response to the stressor. The development of personality within the individual, i.e. its formation through the animal's youth and life experiences, is still somewhat neglected by the animal literature. It has been addressed by research on children (Goldsmith et al. 1987; Rothbart 2007). Late childhood and early adolescence in humans have been found to be key periods in the formation of personality and the kinds of personality displayed (Soto et al. 2011) and although the study could not attribute the cause to neurological development in these critical periods, it remains a possibility. Animals are an ideal

model for studying the neurological basis of personality (Canli & Amin 2002) and as we have so many properly validated tests of fear, and production systems which result in slaughtered animals, this is a potential resource for linking the study of neurology and how fear presents behaviourally in animals. If we were capable of identifying the emotion of fear in neuroimaging (Canli & Amin 2002) it may be possible to obtain a truer measure of fearfulness than behaviours exhibited in response to a fear-eliciting stimuli.

7.5 Future research

This body of work suggests we can use biotelemetry systems to inform us as to how the animal perceives its environment. More work is needed before we can establish reliable methods for predicting personality based on biotelemetry systems however, including work on personality traits not included in this study. The first and most important aspect is that these effects must be established across different herds and in different management systems, such as seasonal calving and outdoor grazing. This does not necessarily involve the repetition of this work in different systems. In systems where biotelemetry databases already exist the models in this study could be used to predict extremes on different traits and these animals could be tested to find if their behaviours in test environments match the prediction. Further personality research in cattle should be aware of the possibility of a behavioural syndrome linking fearfulness and activity and should consider measures of physiology and possibly neurological imaging to identify fear where it is not demonstrated behaviourally.

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9 Appendix

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